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The Cellular and Molecular Basis of Sour Taste

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Abstract

Sour taste, the taste of acids, is one of the most enigmatic of the five basic taste qualities; its function is unclear and its receptor was until recently unknown. Sour tastes are transduced in taste buds on the tongue and palate epithelium by a subset of taste receptor cells, known as type III cells. Type III cells express a number of unique markers, which allow for their identification and manipulation. These cells respond to acid stimuli with action potentials and release neurotransmitters onto afferent nerve fibers, with cell bodies in geniculate and petrosal ganglia. Here, we review classical studies of sour taste leading up to the identification of the sour receptor as the proton channel OTOP1.

INTRODUCTION

Taste allows animals to sample the chemical compositions of possible foods to identify essential nutrients and avoid harmful or toxic substances. In general, taste stimuli are described in terms of five fundamental taste qualities: bitter, sweet, umami, salty, and sour. These taste qualities are encoded by the activation of different subsets of peripheral taste receptor cells (TRCs) that express distinct sensory receptors (1). While receptors for bitter, sweet, and umami were identified more than 15 years ago (2), and the receptor for attractive salty taste was shown to be the Na^+-permeable ENaC channel (3, 4), the receptor for sour taste remained mysterious. This changed in 2018 with the description by Tu et al. (5) of a gene (*Otop1*) that encodes a novel proton channel in taste cells and subsequent studies confirming its direct role in sour taste transduction (6, 7). This review describes the historical antecedents leading up to the identification of the sour receptor and the rapid progress made since.

We start by describing the basic anatomy of the taste system, from sensory cells on the tongue to the first relay station. We then review the history of research on sour taste, starting with psychophysical experiments showing that the perception of sourness is associated with the pH of the stimulus. We also describe early electrophysiological experiments to characterize stimulus response properties of gustatory nerve responses in various animal species. Finally, we describe the cellular-level analyses and molecular genetics that were used to identify cell types and receptors for sour taste. We also describe the many false leads and the impediments that gave rise to premature claims that the sour receptor had been identified. A theme that emerges is the difficulty in identifying a receptor for what is arguably one of the least specific stimuli, the proton. We also describe how the discovery of OTOP1 as a proton channel has had broad implications for ion transport and cellular physiology in a variety of contexts.

TASTE SYSTEM ANATOMY

The peripheral taste system consists of a relatively simple circuit of cells that detect and relay information to the brain (8). The sensory cells, TRCs, are clustered together in taste buds. These cells release neurotransmitter onto afferent nerve fibers with cell bodies in gustatory nuclei that relay information to the nucleus of the solitary tract (NTS) in the brainstem (**Figure 1**).

The Taste Bud

Taste buds are composed of a cluster of 50–100 cells, located on the lingual epithelium, palate, upper esophagus, pharynx, and larynx (9). Within the taste bud, TRCs are closely packed and elongated in shape, sending an apical process to the taste pore, a break in the surface of the epithelium of ~2–10 μm in diameter (10). Taste buds are distributed within three distinct regions of the tongue: Fungiform papillae are dispersed throughout the anterior tongue, while foliate and circumvallate papillae are densely packed in grooves on the sides (foliate) and back of the tongue (circumvallate) (**Figure 1**).

Taste Receptor Cells

Taste cells have been classified into four types (types I–IV), initially based on differences in ultrastructural features evident in electron micrographs (10–12) and now using immunohistochemical and genetic markers. Of the four, two clearly transduce sensory signals (types II and III); the other two are basal precursor cells (type IV) and supportive, glial-type cells (type I), whose functions are still not well understood. Type II cells are required for, and express components of, transduction for sweet, bitter, and umami taste. A convenient marker for this cell type is the ion

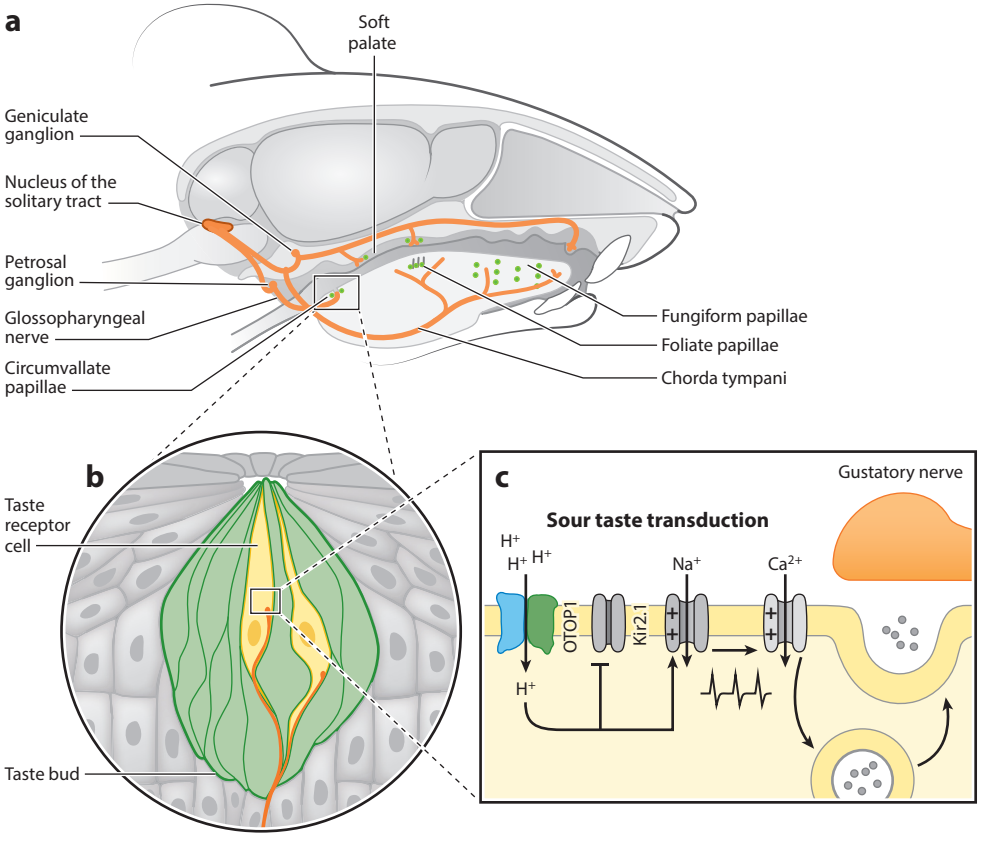

Figure 1

Anatomy and mechanism of sour taste. (*a*) Taste buds and gustatory neurons: Taste buds are shown on the tongue in three distinct zones (circumvallate, foliate, and fungiform papillae) and on the soft palate. Innervation of the anterior tongue by the chorda tympani and posterior tongue by the glossopharyngeal nerve is shown. The gustatory neurons have cell bodies in petrosal and geniculate ganglia and project to the nucleus of the solitary tract. Panel adapted from Reference 119. (*b*) Taste buds are composed of ~50–100 cells, of which ~10% are type III taste receptor cells (TRCs) that mediate the detection of acids that are the stimulus for sour taste. Taste stimuli gain access to TRCs through an opening in the epithelium through which the TRCs project microvilli. Neurotransmitter is released onto gustatory nerve fibers that innervate the TRCs. (*c*) Sour taste transduction is initiated when protons (H^+) enter through OTOP1 proton-selective ion channels, which serve as sour taste receptors. The combination of a direct effect of the entry of positive charge, and indirect effects through inhibition of the Kir2.1 inward rectifier by intracellular acidification, leads to membrane depolarization. With sufficient depolarization, voltage-gated Na^+ channels open to produce action potentials that open voltage-gated Ca^{2+} channels, leading to vesicular neurotransmitter release (at a distance from the OTOP1 channels, but shown in close proximity to the channels for simplicity).

channel TRPM5, which is activated by intracellular calcium downstream of bitter, sweet, and umami receptor signaling (13–15). Other reporters for type II TRCs include PLCB2 and IP3R3 (16, 17).

The type III cells are the primary sensory receptor cells for sour taste and are the focus of this review. They can be identified by expression of a unique set of signaling molecules that include PKD2L1 (18–24), a TRP channel whose function in taste is not known (see below). Other reporters for type III TRCs include Gad67, SNAP-25, PGP-9.5, NCAM, 5-HT, and CgA (25–31).

Gustatory Nerves

Sensory neurons with cell bodies in the geniculate, petrosal, and trigeminal ganglia innervate the oral cavity and carry information to the NTS (**Figure 1**). The chorda tympani [a branch of the facial nerve (VII)] and the glossopharyngeal nerves (IX) innervate the anterior third and posterior tongue, respectively, while the greater superficial petrosal nerve innervates the soft palate, and the superior laryngeal nerve innervates the larynx and epiglottis (32, 33). There are also free nerve endings that extend around the taste bud that relay thermal and tactile information (34). Thus, recordings from the chorda tympani nerve (or geniculate ganglion) can provide information about sensory signals originating in fungiform papillae, while recordings from the glossopharyngeal nerve (or petrosal ganglion) provide information about signals coming from circumvallate and foliate papillae.

Taste Maps and Regional Specialization

Early studies suggested that different areas of the tongue were responsible for detecting distinct basic tastes, creating a taste map of the tongue (35). This taste map has since not been substantiated, and taste receptors are, for the most part, expressed in all taste regions (36). One exception is amiloride-sensitive salt taste, which is restricted to the anterior tongue (37). There are also reported differences in expression of signaling molecules in type III cells from different regions of the tongue (22). For example, PKD1L3 (which assembles with PKD2L1; see below) is found only at the back of the tongue (19). Although the functions of the different populations of taste buds are not well understood, an attractive hypothesis is that different parts of the tongue play distinct roles in ingestive behavior.

A SHORT HISTORY OF RESEARCH INTO SOUR TASTE

Human Psychophysics

The first description of the physical-chemical basis for sour taste comes from chemists and psychologists working in the late nineteenth and early twentieth centuries, coinciding with new ways of measuring the concentration of hydrogen ions in solutions. A Danish scientist, S.P.L. Sorenson, is credited with introducing the pH scale in 1909, and a commercial pH meter was introduced in 1934 by Arnold Beckman, greatly facilitating the study of sour taste. Taste tests of pure chemicals revealed that hydrochloric acid (HCl) tasted sour (and a little bitter and astringent) (38, 39), with a reported threshold of ~n/1,000 (one gram/1,000 liters, corresponding to a pH of ~4.56). Organic acids, including citric, tartaric, and malic acids, were also noted to taste sour in proportion to their pH, providing the first evidence that protons are required to elicit sour taste.

But it was soon noted that weak (organic) acids taste more sour than predicted based solely on the concentration of the hydrogen ion (40). Weak acids do not dissociate completely like strong acids, such as HCl, but exist in an equilibrium containing a mixture of free H^+ ions (bound to water as hydronium, H_3O), free anions, and the H^+-bound anions (e.g., $H^+ + Ac^- \rightarrow HAc$). Thus, one explanation for the enhanced sourness of weak organic acids was that they contained a larger number of bound protons that could be released by mass action into the solution as the protons (or free acid) are consumed (e.g., by binding to receptors or buffers or by uptake into cells). While total acidity, or normality, the sum of the bound and free hydrogen ions in solution, was initially proposed as a predictor of sour taste (39), this theory did not stand up to further scrutiny using a large panel of acids (41–44). An alternative explanation posited that it was the ability of weak acids to cross cell membranes that made them taste more sour (45–47). This question remains unanswered.

Gustatory Nerve Recordings

Starting in the 1930s, methods to record from cranial nerves in an intact animal were applied to the taste system (48, 49). The electrical activity from the gustatory nerves that innervate the tongue was measured with an extracellular electrode and processed to yield an integrated nerve response (50). Recordings from the chorda tympani showed that the gustatory system responds to HCl with a threshold of 0.001 N (~pH 4) and that responses increase in intensity up to a concentration of 0.1 N (pH 1), at which point the acid becomes tissue damaging (51). Similar sensitivity to acids was observed across several species (cat, rat, and rabbit) that otherwise show variation in sensitivity to other taste stimuli related to dietary niche. This suggests that the gustatory sensitivity to acids is an evolutionary adaptation that benefits a large range of species.

Recordings from single gustatory nerve fibers (axons) from a variety of species have shown that single units that are sensitive to acids tend to be heterogeneous in their response profiles (52). Some units respond only to acids, while others respond to additional stimuli, including those that taste salty or bitter but never sweet (53). This observation could be explained if multiple types of TRCs were innervated by single fibers, or if the TRCs themselves were tuned to multiple stimuli (54, 55).

A consistent observation, regardless of species or nerve studied, is that weak acids evoke a larger response than strong acids at the same pH (56, 57). Further, the magnitude of the gustatory nerve response to weak acids is correlated with the degree of intracellular acidification of the TRCs (58), raising the possibility that cellular acidification represents a key step in sour taste transduction. We discuss this topic in more detail later. Importantly, integrated gustatory nerve recordings have been used, as described below, to test contributions of candidate molecules to sour taste detection, and this method is considered the gold standard for in vivo validation of putative taste receptors.

Patch-Clamp Recordings

The first recordings from TRCs proper, using cells from the mudpuppy *Necturus* and frog, showed that they are electrically excitable and can fire action potentials (59–61). This surprising result reversed the prevailing dogma that taste cells, like photoreceptors, respond to sensory stimuli with graded changes in membrane potential (62). Subsequent studies of mouse TRCs showed that while both type II and type III TRCs fire action potentials, they differ substantially in electrophysiological properties (63). For example, type III (but not type II) cells express voltage-gated calcium channels (64), which allow an influx of calcium necessary for vesicular neurotransmitter release. Importantly, the restricted expression of voltage-gated calcium channels to type III cells has made it possible to identify type III TRCs based on an observed calcium elevation in response to K^+ depolarization. It is worth noting that the neurotransmitter used by type III cells has not been definitively identified, although there is evidence for contributions from both serotonin and ATP (65).

Early patch-clamp studies also attempted to measure the sensory responses of TRCs to acid stimuli. Acids elicited membrane depolarization in a subset of TRCs from the mudpuppy through inhibition of apically located, outwardly rectifying K^+ channels (66, 67). Other studies showed evidence for proton permeation through amiloride-sensitive ion channels in TRCs from rodents and proposed this as a mechanism for sour transduction (68–70). However, these responses were not shown to be associated with type III TRCs and may in fact be due to a low level of nonspecific proton flux through amiloride-sensitive Na^+ channels that mediate salty taste. At the same time, Okada et al. (71) reported that acetic acid activated a cationic conductance in frog TRCs and later that acids evoked an NPPB [5-nitro-2-(3-phenylpropylamino)benzoic acid]-sensitive Cl^- conductance in mouse TRCs (72). Although conceptually sound, these experiments all suffered

from the same technical limitation: They were not able to genetically or functionally single out sour TRCs.

pH and Calcium Imaging

A separate approach adopted early on was to measure responses of taste cells with calcium or pH imaging. In one series of experiments, the intracellular pH of TRCs in intact taste buds was found to steeply track with extracellular pH, suggesting a specific proton influx pathway (58, 73). One limitation of these experiments was that they lacked cellular-level resolution. To overcome this limitation, other groups used confocal imaging of lingual slices loaded with calcium indicators (74). These experiments showed that a subset of taste cells, functionally identified as type III TRCs, responded to focal application of the acid stimuli (100 mM citric acid or 10 mM HEPES-buffered saline titrated to pH 1.5, 3.0, or 7.0). The same stimuli caused widespread acidification of the taste bud, which was more pronounced in response to citric acid than HEPES-buffered stimuli. By identifying the subtypes of cells that responded to acid stimuli and ruling out candidate receptor mechanisms, these studies laid the groundwork for future experiments to identify the sour receptor.

THE SEARCH FOR THE SOUR RECEPTOR: FALSE STARTS

The identity of the sour receptor has been the subject of intense investigation for the last 50 years. That there existed a protein receptor was not a foregone conclusion given the nonspecific nature of the stimulus. Indeed, in 1972 it was concluded that "sour taste is induced by binding of hydrogen ion to a phosphate group of phospholipids in the gustatory receptor membrane" (75, p. 459). This view was, however, not widely accepted, and over the years, at least three membrane proteins were proposed to function as the sour receptor.

ASIC

The acid-sensing ionic channel (ASIC), a relative of amiloride-sensitive Na channels, was an ideal candidate to function as a sour receptor (76). Shortly after the first ASIC was cloned (77), a second ASIC that was 67% identical to ASIC1 (called MDEG1, but now known as ASIC2) was identified from a rat circumvallate papillae cDNA library (78). ASIC2 conducted inward Na^+ currents in response to mildly acidic stimuli and appeared to localize to a subset of TRCs, leading to the claim that MDEG1/ASIC2 was the sour receptor (78). Subsequent experiments, however, showed no effect of amiloride on gustatory taste responses (79), no effect of genetic inactivation of ASIC2 on the response of isolated TRCs to acidic stimuli (80), an absence of amiloride-sensitive ASIC channels in patch-clamp recordings from type III TRCs (23, 24), and no evidence for ASIC2 mRNA in type III cells (81; E. Liman, unpublished data). Thus, ASICs are no longer thought to contribute to the gustatory response to acids.

HCN4

The second candidate receptor for sour taste was the hyperpolarization-activated and cyclic-nucleotide-gated channels HCN1 and HCN4 (82). Hyperpolarization-activated currents sensitive to acidic solutions were detected in a subset of TRCs from rat that also responded to acid stimuli with inward currents. Immunolabeling showed clear expression of HCN1 and HCN4 in a subset of cells distinct from those that express gustducin, a marker for type II cells. Subsequent experiments confirmed the localization of HCN4 in type III TRCs (83) and the presence of mRNA

encoding HCN4 in type III TRCs (81; E. Liman, unpublished data). However, there is no evidence that hyperpolarization-activated channels are essential for sour taste transduction (74), and they may instead function to modulate responses to acids.

PKD2L1

When PKD2L1, a member of the TRP family of ion channels, was identified by three groups simultaneously as highly enriched in type III TRCs (18–20), it was widely hailed as the elusive sour receptor. Expression of PKD2L1 is restricted to cells that express markers for type III TRCs (21), and it appears to be localized to the apical surface of the cells (19). In circumvallate, but not fungiform papillae, PKD2L1 is coexpressed with PKD1L3, a structurally unrelated membrane protein that promotes its surface expression (19). Although initially proposed to be activated by acids, subsequent studies showed that the PKD2L1/PKD1L3 channel responds to the removal of acids (84).

Despite the enthusiasm with which the discovery of PKD2L1 was greeted, it has not proven to be essential for sour taste transduction. Two groups working independently showed no, or only minor, changes in gustatory nerve responses in animals with a targeted inactivation of PKD1L3 or PKD2L1 or in a double knockout (85, 86). Similarly, cellular responses to acid stimuli were not eliminated in *PKD2L1*$^{-/-}$ animals, although an attenuation of acid responses was noted (86). Moreover, as described below, inward Na^+ currents, such as could be mediated by PKD2L1/PKD1L3, are not detected in type III cells in response to acid stimuli (24). Thus, at present, there is insufficient evidence to support a role for PKD2L1 in acid sensing by type III TRCs, and its function in these cells remains a mystery.

It is worth noting that PKD2L1 is also expressed in the spinal cord (18). These PKD2L1-expressing spinal neurons (known as cerebrospinal fluid–contacting neurons) respond to acids through the activation of ASICs, bearing little relationship to the sensory response of type III taste cells (see below) (23). The function of PKD2L1 in such disparate cell types is not understood, but recent evidence suggests a role in mechanosensation (87).

THE SENSORY CELLS FOR SOUR TASTE

Type III Taste Receptor Cells Transduce Sour Tastes

The identification of molecular markers for the cells that detect sour tastes was the culmination of decades of research. The identification of type III TRCs as the cells that mediate sour taste was based on two lines of evidence, one showing that type III TRCs are necessary for the detection of acids by the taste system, and the other showing that they display a specific sensitivity to acids.

To show that type III TRCs are necessary for sour taste, Huang et al. (18) genetically ablated these cells by expressing an attenuated form of diphtheria toxin under the *PKD2L1* promoter. In the absence of *PKD2L1*-expressing type III TRCs, the chorda tympani nerve response to sour stimuli (HCl, acetic acid, and citric acid) was largely eliminated, whereas responses to bitter, sweet, umami, and salty stimuli were not affected (18). A similar strategy was used to show that type III TRCs are necessary for the taste of carbonation, "water taste," and a component of the gustatory response to high salts (88–90).

Evidence that type III/*PKD2L1*-expressing TRCs respond specifically to acids comes from recordings made from genetically identified TRCs. Yoshida et al. (31) showed that apical application of acids, starting at a pH of ~3, elicited action potentials in GFP^+ cells of intact fungiform taste buds from *GAD67-GFP* mice. Similar results were found in dissociated YFP^+ TRCs of circumvallate papillae from *PKD2L1-YFP* mice (6, 23, 24) (**Figure 2**). A difference in pH sensitivity

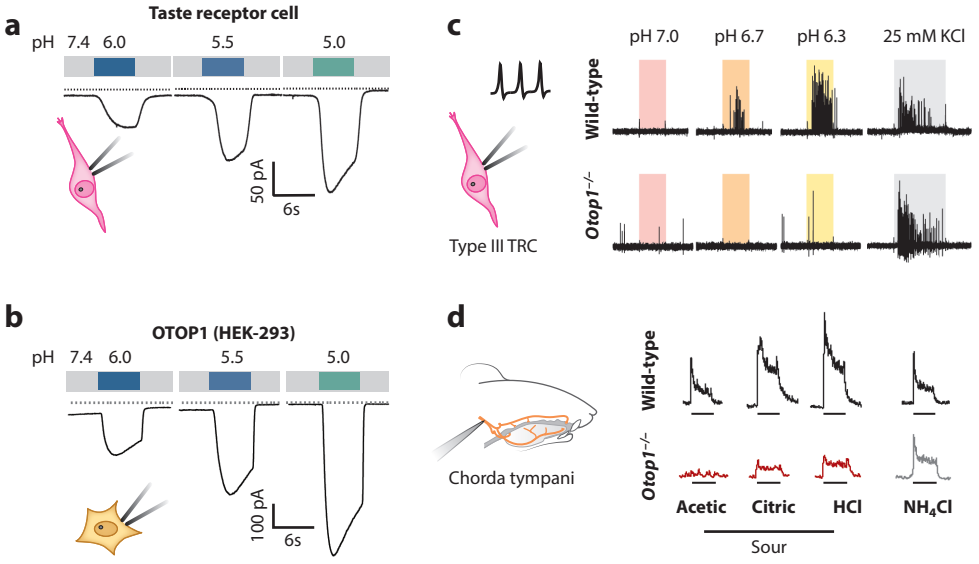

Figure 2

OTOP1 is the sour receptor. (*a*) Patch-clamp recording from a type III taste receptor cell (TRC; from a *PKD2L1-YFP* mouse) showing that acid stimuli evoke inward currents that increase in magnitude as the pH is lowered. These currents are evoked in the absence of extracellular Na^+ and are carried by H^+. (*b*) Patch-clamp recording from an HEK-293 cell expressing OTOP1. Inward proton currents evoked in response to acid stimuli are similar to those observed in type III TRCs (panel *a*). (*c*) Cell-attached patch-clamp recording from type III TRCs. Action potentials are evoked in response to acid stimuli in cells from a wild-type mouse. No action potentials are observed to the same stimuli in recordings from *Otop1*$^{-/-}$ mice, which respond to the positive control, potassium chloride (KCl). (*d*) Gustatory nerve recording from a wild-type and *Otop1*$^{-/-}$ mouse shows that *Otop1* is required for the response to acids. NH_4Cl serves as a control. Responses are normalized to baseline. Figure adapted with permission from Reference 6; copyright 2019, Elsevier.

of the cells in the two studies, with isolated cells responding to a more mild acidification (pH 6), may be attributed to the larger surface area of the isolated cell in contact with the acid stimuli (23).

Proton Currents in Type III Taste Receptor Cells

In 2001, Lyall et al. (58, p. C1005) wrote that "intracellular pH is the proximate stimulus in sour taste transduction." Definitive evidence that sour taste cells have a cell type–specific mechanism to allow proton entry did not come until a decade later (24). In patch-clamp recordings from *PKD2L1-YFP* TRCs, Chang et al. (24) found that lowering the extracellular pH induced an inward current that increased in magnitude as pH was lowered (**Figure 2**), which was not observed in type II TRCs. The acid-activated current was not sensitive to changes in concentrations of Na^+, Ca^{2+}, K^+, or Cl^-, and its reversal potential (the voltage at which the current reverses direction from inward to outward) changed at ~59 mV/pH. This indicated that it was carried by protons. Consistent with this interpretation, type III, but not type II, TRCs showed a rapid drop in intracellular pH in response to lowering the extracellular pH (pH tracking). In an effort to identify blockers for this novel proton current, Chang et al. found that it was insensitive to amiloride, ruling out a contribution from ENaC and ASIC channels, and was instead inhibited by Zn^{2+} (23, 24), a relatively nonspecific blocker of voltage-gated proton channels and other proton transport mechanisms (91, 92).

Finally, to test whether the proton current plays a role in sensory transduction, Chang et al. (24) delivered protons to the apical surface of type III TRCs through ultraviolet-uncaging of caged protons. Apical protons elicited action potentials in the absence of apical Na^+, a response that was blocked by millimolar concentrations of Zn^{2+}. Together, these experiments described a novel proton-selective current specific to type III TRCs, likely to mediate sour transduction (**Figure 1**), that bore no resemblance to currents described in any other cell type or carried by any known ion channel (24).

K^+ Channels Sensitive to Intracellular Acidification: A Role in Amplifying Sensory Signaling?

The identification of a specific Zn^{2+}-sensitive proton current provided one mechanism for proton entry into type III TRCs, but it is well known that weak acids can penetrate cell membranes in the absence of a specific transport mechanism. Indeed, a leading explanation for why weak acids taste more sour than strong acids has been that cellular acidification directly engages the machinery for cellular excitation and transmitter release (93). To test this, Ye et al. (94) measured the response of TRCs (from *PKD2L1-YFP/TRPM5-GFP* mice) to weak acids applied at neutral pH. Type III, but not type II, TRCs responded robustly to the weak acids. Using a combination of transcriptome profiling, pharmacology, and cell type–specific knockout mice, they showed that weak acids inhibited an inward rectifier K^+ channel Kir2.1, which is sensitive to intracellular pH and maintains the resting potential in type III cells (94). Interestingly, the resting potential in type II cells is also set by Kir2.1 channels, but a higher level of expression of Kir2.1 channels in type II TRCs makes them less sensitive to the blocking effect of intracellular acidification (94).

It is worth noting here that type III TRCs also express high levels of two-pore domain (K2P) channels, including TWIK-1 (KCNK1) and TASK-2 (KCNK5) (94–96). K2P channels may play a role in setting the resting potential in some cell types, and some isoforms are inhibited by extracellular or intracellular acidification (97). However, a role in the cellular physiology of type III TRCs has yet to be established (94, 96).

Based on these results, it is reasonable to propose that Kir2.1 acts downstream of the entry of protons through a proton-selective ion channel and that inhibition of Kir2.1 by intracellular protons serves to amplify the sensory response (**Figure 1**). Kir2.1 may also play a role in sensitizing taste cells in the presence of weak acids, thereby enhancing their perceived intensity. It is worth noting that further evidence for a role of Kir2.1 in taste transduction cannot be obtained by recording electrical activity or behavior from a knockout or conditional knockout of Kir2.1, as this is expected to blunt or eliminate all electrical signaling in the taste cells.

DISCOVERY OF OTOP1 AS A PROTON CHANNEL AND CANDIDATE SOUR RECEPTOR

The many years of searching for the sour receptor came to fruition with the identification of OTOP1 as a candidate sour receptor by Tu et al. in 2018 (5) and subsequent evidence that it is required for sensory responses in isolated cells and intact animals (6). Starting from the premise that the sour receptor would be a novel proton channel, Tu et al. (5) performed transcriptome profiling (RNAseq) of TRCs, searching for genes enriched in sour type III cells that encoded transmembrane proteins of unknown or poorly understood function. They then tested 43 candidates for functional activity when expressed in *Xenopus* oocytes. One candidate, encoding the predicted transmembrane protein OTOP1, induced large currents in response to lowering the extracellular pH. OTOP1 currents, measured in either *Xenopus* oocytes or HEK-293 cells,

increased in a dose-dependent manner in response to lowering the extracellular pH (**Figure 2*b***). The ability of the *Otop1* gene to induce expression of acid-induced currents in two different cellular contexts indicated that OTOP1 encoded an essential subunit of a novel ion channel.

Further characterization of OTOP1 showed that it forms an ion channel that is exquisitely selective for H^+ over Na^+ by a factor of more than 10^5 and impermeable to other monovalent ions (K^+, Cs^+, Li^+), divalent cations (Ca^{2+}), and anions (Cl^-) (5). The lack of dependence on other ions (and specific solutes) argues strongly that OTOP1 is a H^+ channel, not a H^+ transporter. OTOP1 is inhibited by Zn^{2+} in a dose- and pH-dependent manner, similar to native proton currents in taste cells (6); the pH dependence of the inhibition likely reflects the competition between Zn^{2+} and H^+ for a common binding site, such as the carboxylate side chain of an amino acid (6, 7).

Otop1 is predicted to encode a protein with 12 transmembrane domains bearing no homology to other ion channels or transporters (98). It is a member of an evolutionarily conserved family of genes that in most vertebrates includes two other members (*Otop2* and *Otop3*). There are three orthologs in the *Drosophila* genome that diverged from a common ancestor, and eight orthologs in *Caenorhabditis elegans* (99). Otop2 and Otop3 in the mouse also form proton channels, as does one of the *Drosophila* orthologs (5).

The function of OTOP1 in the taste system is described below. The function and distribution of the OTOP channels in other systems is still poorly understood. *Otop1* was first identified as a gene expressed in the vestibular system, the mutation of which caused a vestibular disorder (100). Mice with mutations in *Otop1* (*tlt* or *mgl*) show an impaired ability to right themselves on a forced swim task; this is attributed to the degeneration of otoconia (101), calcium carbonate crystals that sit on top of the vestibular hair cells and are required for the sensation of gravity and acceleration. Zebrafish with mutations in *Otop1* show similar phenotypes (102). *Otop1* is also expressed in adipose tissue (103), while *Otop2* is expressed in the colon (104–106). The function of OTOP channels in these systems remains poorly understood.

OTOP1 IS THE SOUR RECEPTOR

Upon its identification as a proton channel in TRCs (5), OTOP1 was considered a promising candidate for the long-sought sour receptor (107). To conclude that OTOP1 is a sour taste receptor, at least two conditions need to be met: (*a*) It is sufficient to bind or otherwise interact with the ligand (protons) such that this interaction leads to an electrophysiological response and/or transmitter release; and (*b*) it is necessary for the sensory response, measured at the sensory receptor cells. Other evidence, such as the match between functional properties of the receptor and human psychophysics, although not essential for categorization as a sensory receptor, can be important to establish relevance to humans and understand the physical underpinning of psychophysical phenomena.

OTOP1 Is Sufficient to Form a Sour Receptor in Heterologous Cell Types

As described above, OTOP1 functions as a proton-selective ion channel when expressed in heterologous cell types, with properties consistent with a role as a sour receptor (5). Indeed, in a more detailed analysis, Teng et al. (6) found that functional properties of heterologously expressed OTOP1 and of native proton currents in taste cells were remarkably similar (**Figure 2**). For example, the IC_{50} (half-maximal inhibitory concentration) for inhibition by Zn^{2+}, and pH dependence of Zn^{2+} inhibition, are nearly the same. This makes it likely that OTOP1 functions as a homodimer (see below) to mediate sour taste transduction. Using a different approach, Zhang et al. (7) ectopically expressed OTOP1 under the promoter for a sweet receptor and showed that sweet-sensitive

gustatory neurons (that innervate the taste cells) acquired sensitivity to acids; these experiments did not, however, show that OTOP1 conferred sensitivity to acids on the sweet-sensitive TRCs. In summary, evidence from heterologous systems shows that as a proton channel, OTOP1 can report the concentrations of extracellular protons, generate a depolarizing response to acids, and serve as a sour receptor (5, 6).

OTOP1 Is Necessary for Sensory Response to Acids by Taste Cells In Vitro and In Vivo

The necessity of OTOP1 for sour taste has been tested by two labs using mice in which the *Otop1* gene was inactivated with CRISPR-Cas9 gene editing ($Otop1^{-/-}$) (6, 7). Both showed that OTOP1 is essential for sour taste. Behavioral effects of *Otop1* gene inactivation are more complicated.

Proton currents. Inward proton currents are not observed in type III TRCs from $Otop1^{-/-}$ mice (6) and are strongly attenuated in magnitude in the *tlt* mutant (a trafficking mutation of *Otop1*) (5, 108). This demonstrates that OTOP1 carries the proton current in taste cells and that OTOP2 and OTOP3, which are expressed at low levels in wild-type TRCs (5), do not compensate for the loss of OTOP1 in $Otop1^{-/-}$ mice. This is true for TRCs from both circumvallate and fungiform papillae, which vary in their expression of other signaling molecules.

pH tracking. pH tracking refers to the observation that the intracellular pH of the TRCs changes with the pH of the extracellular solution. In type III TRCs, pH tracking has long been thought to reflect the initial step in sensory signaling. Type III TRCs from $Otop1^{-/-}$ mice show only nonspecific responses to changes in extracellular pH, similar to those observed in (nonsour) type II TRCs (6). Thus, pH tracking in type III TRCs is dependent on *Otop1*.

Action potentials in taste receptor cells. As described above, type III TRCs respond to acid stimuli with trains of action potentials that encode stimulus intensity. These responses are largely eliminated in type III TRCs from $Otop1^{-/-}$ mice (6) (**Figure 2*c***). A small residual response (one or two action potentials) can still be observed in $Otop1^{-/-}$ cells to suprathreshold stimuli and may reflect proton leakage through nonspecific pathways.

Response of gustatory neurons and nerves. Two groups showed independently that gustatory nerve responses to acid stimuli delivered to the tongue or oral cavity were strongly, and specifically, attenuated in $Otop1^{-/-}$ mice. This was true in recordings from both the chorda tympani and glossopharyngeal nerves (6, 7) (**Figure 2*d***). Interestingly, responses to weak and strong acids were similarly attenuated, arguing against a separate pathway for the detection of weak acids. Both groups reported a residual response to acids in $Otop1^{-/-}$ mice; this response has been attributed to activation of bitter receptors, based on its sensitivity to allyl isothiocyanate (AITC) (7, 90). A similar conclusion was reached in calcium imaging of taste-induced responses of gustatory neurons (7). In wild-type mice, ~10% of gustatory neurons responded to the sour taste stimulus (50 mM citric acid), while no responsive neurons were detected in $Otop1^{-/-}$ mice.

Thus, multiple lines of evidence from two groups show that OTOP1 is required for taste responses to acid stimuli in isolated cells and intact animals.

Behavioral responses to acids. Given the compelling evidence that OTOP1 functions as a sour receptor, it might be expected that the $Otop1^{-/-}$ mice would be indifferent to, or at least less sensitive to, sour stimuli in assays of taste preference. Mice find acid stimuli aversive, which can be

assessed with two bottle taste preference or brief access tests (lickometer) (109, 110) in animals that are water deprived. No difference in sensitivity to citric acid (6, 7) or HCl (6) between wild-type and *Otop1*$^{-/-}$ mice was observed. These results are consistent with results showing that behavioral aversion to acids is unchanged in mice completely lacking the type III TRCs, or in which synaptic transmission from type III TRCs is disrupted (89, 111).

The aversiveness of acids, therefore, may be partly mediated by acid-sensitive nociceptive afferents that innervate the oral cavity and larynx. Consistent with this possibility, the ablation of trigeminal neurons (through direct injection of the toxin resiniferatoxin bilaterally into both nuclei) in *Otop1*$^{-/-}$ mice, but not wild-type mice, strongly attenuated behavioral aversion to acids (7). Currently, the receptors responsible for the trigeminal response to acids are not known, as nociceptors express several ion channels sensitive to extracellular or intracellular acidification, including TRPV1, TRPA1, and ASIC2 (112–115). The observation that disruption of either nociceptive signaling or *Otop1* (6, 7, 116) does not change behavioral aversion to acids suggests that there is an impressive degree of redundancy in this system.

STRUCTURE AND FUNCTION OF THE SOUR RECEPTOR OTOP1

Shortly after OTOP1 was shown to form a proton channel, its structure was solved by cryogenic electron microscopy (cryo-EM). One group reported the structure of zebrafish OTOP1 (117), while structures of the related protein OTOP3 (from chicken and *Xenopus*) were reported simultaneously by two labs (117, 118). Several major insights can be gleaned from these studies. First, they show that OTOP1 (and OTOP3) assembles as a homodimer. The 12 transmembrane helices form two structurally similar domains: an N domain that comprises the first six transmembrane helices and a C domain comprising the remaining six helices. This leads to a pseudotetrameric structure, with the four pseudosubunits adopting a barrel-shaped fold surrounding a central cavity (**Figure 3**).

Unlike other ion channels, the central cavity in the OTOP channels is filled with lipids and cannot support ion transport (117). Instead, three possible permeation pathways were identified, each of which contains a water-filled entrance leading to a constriction or a hydrophobic plug.

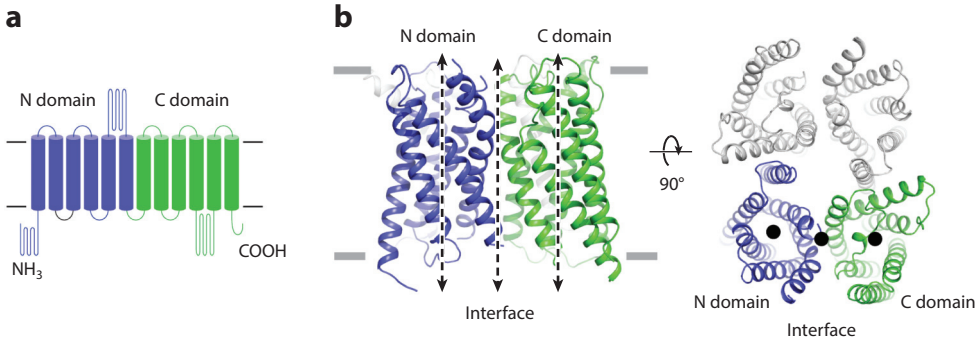

Figure 3

Structure of OTOP1. (*a*) OTOP1 contains 12 transmembrane domains, with N and C termini located intracellularly. The structure shows that the protein can be divided into two domains (N and C) that adopt similar structures (see panel *b*). (*b*) Cryogenic electron microscopy (cryo-EM) structure of zebrafish OTOP1 shows that the protein assembles as a dimer, with a cholesterol-filled central cavity. Three possible proton permeation pathways have been identified. Panel *b* courtesy of K. Saotome and A. Ward and based on zfOTOP1 (Protein Data Bank: 6NF4) in Reference 117.

Protons, unlike other ions, do not need to diffuse but instead can hop along water wires—chains of H_2O that share hydrogen bonds. Permeation of protons through OTOP channels likely involves movement along an aqueous pathway, as well as through hydrogen bonds in the protein. Presently, it is not clear which of the three permeation pathways mediates the observed proton flux through the channels, and it is possible that there is more than one pathway utilized, each having unique characteristics. It is also not clear if the solved structures of OTOP channels are in the open or closed states, as very little is currently known about the gating of these newly described channels.

CONCLUSION

Thus, the long search for the sour receptor has led in a short time, from the identification of OTOP1 as a proton channel and candidate sour receptor, to evidence from *Otop1*$^{-/-}$ mice that OTOP1 functions as a sour receptor, to the publication of its structure. This information and these new tools will surely usher in a new era in the study of sour taste.

SUMMARY POINTS

1. Sour taste is detected by a subset of taste receptor cells, type III cells, that express a distinct set of molecular markers.
2. Type III cells express a novel proton channel encoded by the *Otop1* gene.
3. The OTOP1 ion channel is perfectly selective for protons and conducts an inward depolarizing current in response to acidic (sour tasting) stimuli.
4. The sour taste responses are dependent on a functional OTOP1 protein.
5. The pain and gustatory systems can each detect ingested acids over a similar pH range, and only when both systems are silenced are aversive behavioral responses to acids attenuated.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Contents

Errata

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