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COMMENTARY

Otopetrin-1: A sour-tasting proton channel

I. Scott Ramsey and John A. DeSimone

Tight control of intracellular pH (pH_i) is fundamental to cellular homeostasis. Liman and colleagues' discovery of a new family of H^+-permeable ion channels conserved from nematodes to insects and man (see Tu et al.) represents an exciting new development. The work is likely to identify new roles for proton transfer in biology, explain long-standing mysteries concerning vestibular function and sour taste transduction, and challenge researchers to elucidate the mechanism of H^+ permeation in a new set of integral membrane proteins. Cells use a variety of integral membrane proteins to monitor changes in pH_o, both directly (via changes in pH_i) and indirectly (via activation of signaling cascades). These include proton-permeable ion channels, adenosine triphosphate–driven H^+ pumps, secondary active transporters and exchangers, light-gated opsins, and pH-sensitive receptors (DeCoursey, 2003). Using expression profiling in murine sour taste cells to identify Otopetrin-1 (Otop1) as a proton channel, Tu et al. (2018) show that heterologous overexpression of related proteins from human, mouse, and *Drosophila melanogaster* is sufficient to confer an ensemble of novel H^+-permeable conductances.

Responses to sour (and salty) tastants use sensing and transduction mechanisms that are distinct from bitter, sweet, and savory (umami), which are detected by T1R- or T2R-family G protein–coupled receptors. These receptors signal through phospholipase Cβ2 to activate TRPM5, a cation-permeable channel that causes membrane depolarization and neurotransmitter release (Huang et al., 2006). In contrast, sour taste responses to both strong (e.g., hydrochloric acid) and weak (e.g., citric and acetic acids) sour stimuli begin with a decrease in pH_i (Chang et al., 2010; DeSimone et al., 2011, 2015). One or more H^+-selective channels that mediate steady-state inward currents are therefore likely to be required for sour taste responses to strong acids. TRPM5 is not expressed in sour taste cells, and PKD2L1, a marker of sour taste cells, is also not the transducer required for transduction of either strong or weak sour taste responses (Horio et al., 2011; DeSimone et al., 2012).

To identify new candidate H^+ channels, Tu et al. (2018) used RNA sequencing to compare gene expression profiles in single taste cells that exhibited Zn^{2+}-sensitive, inwardly rectifying proton currents (but not TRPM5-GFP$^+$ or its current). From this, they identified candidate genes whose expression was enriched in PKD2L1-YFP$^+$ cells and that were predicted to encode integral membrane proteins of unknown function. Surprisingly, of the 41 cDNAs tested, only Otop1 fulfilled the criteria expected for the sour taste proton channel. Upon expression of Otop1 in *Xenopus laevis* oocytes or mammalian HEK-293 cells, current reversal potentials measured using voltage-clamp electrophysiology and pH-sensitive fluorescent dye changes demonstrated that protons permeate the membrane. For example, pH_i decreases rapidly when pH_o is lowered, and the potency for current block by Zn^{2+} (IC_{50} = 200 µM in mOtop1 at pH_o 5.5) is similar to the native H^+ current in sour taste cells (Chang et al., 2010; Bushman et al., 2015). Tu et al. (2018) further showed that Otop1 is expressed in PKD2L1-YFP$^+$ (but not TRPM5-GFP$^+$) mouse circumvallate taste cells and that proton currents in taste cells from Otop1 mutant mice are significantly smaller than those from WT. Together, the new data present a compelling argument that Otop1 forms an entirely new kind of proton-selective ion channel protein.

Because protons are fully dissociated only for strong acids, the entry mechanisms for acid equivalents across the taste cell apical membrane are likely to be distinct for weak and strong acids. In rats and mice, chorda tympani (CT) neural responses to HCl are modulated by voltage applied across the lingual surface and blocked by divalent cations including Zn^{2+}, whereas acetic acid responses are affected by neither voltage nor Zn^{2+} (DeSimone et al., 2012, 2015). Micromolar $[Zn^{2+}]$ is well established as a blocker of the H^+ conductance in sour taste cells, and the proton entry mechanism for strong acids is therefore hypothesized to require a proton channel protein (Chang et al., 2010; DeSimone et al., 2011, 2015). Otop1 represents the best candidate yet for the sour taste receptor, and the development of genetically modified animals lacking Otop1 will undoubtedly enable a direct test of this hypothesis in the near future.

Intracellular acidification resulting from weak acid stimuli triggers an increase in intracellular-free Ca^{2+} ($[Ca^{2+}]_i$) via release from intracellular stores and/or influx through channels in the

Department of Physiology and Biophysics, School of Medicine, Virginia Commonwealth University, Richmond, VA .

Correspondence to Ian Scott Ramsey: Ian.Ramsey@vcuhealth.org.

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basolateral membrane that drives neurotransmitter release (Richter et al., 2003; Fig. 1). The membrane-permeable Ca^{2+} chelator BAPTA-AM abolishes CT responses to both strong and weak acids, and subsequent addition of the Ca^{2+} ionophore ionomycin restores neural activity (DeSimone et al., 2012). The effect of extracellular acetic acid to lower pH_i is tightly linked to an increase in $[Ca^{2+}]_i$ (DeSimone et al., 2012), but the targets of intracellular H^+ require further elaboration. CT nerve responses to HCl in WT mice are enhanced by H_2O_2, and the Zn^{2+}-sensitive CT response to HCl is 64% smaller in $gp91^{phox}$-null mice, suggesting a role for nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in controlling proton channel function or localization (DeSimone et al., 2012). NADPH oxidase and the voltage-gated proton channel Hv1 control pH_i and $[Ca^{2+}]_i$ responses in leukocytes that mount a respiratory burst of superoxide anion production (Morgan et al., 2009; Ramsey et al., 2009; El Chemaly et al., 2010), and similar mechanisms could be at play in sour taste cells.

The work of Tu et al. (2018) is likely to lead to the development of genetic and pharmacological tools that will clarify additional lingering questions about the nature of H^+ conductances in vestibular function and taste transduction. Naturally occurring Otop1 mutations (*tlt* and *mlh*) cause vestibular defects, presumably by altering Otop1 trafficking (Hurle et al., 2003; Kim et al., 2011). The smaller currents (but evidently similar pH dependence and Zn^{2+} sensitivity) that result from *tlt* (A151E) and *mlh* (L408Q) expression in oocytes are consistent with the idea that surface expression is compromised (Tu et al., 2018). However, more studies are needed to rule out other possible explanations, and behavioral tests involving sour taste in *tlt* and *mlh* mice remain to be performed. Experiments designed to elucidate whether and how otopetrins are modulated by a host of posttranslational modifications, including by reactive oxygen and nitrogen species, second messengers, bioactive lipids, phosphorylation, etc., could prove useful in understanding biological variation in behavioral responses to sour tastants.

Whether otopetrins will prove to be necessary for taste transduction of weak acid stimuli is less clear. Cl^- permeability in Otop1 is five orders of magnitude lower than H^+ (Tu et al., 2018), suggesting that Otop1 is likely to be impermeable to dissociated anions like acetate and citrate. Although Otop1 transfection dramatically potentiates pH-sensitive fluorescent dye responses to a drop in pH_o, robust responses to acetic acid can be measured in nontransfected HEK-293 cells and therefore do not require Otop1 (Tu et al., 2018). The intensity of CT responses to both strong and weak acids depends on a decrease in pH_i, so weak acid responses are expected to require shuttling of the neutral form followed by intracellular dissociation (DeSimone et al., 2012, 2015). The robust response of weak acids in HEK-293 cells suggests that weak acid shuttle mechanisms may not be specific to sour taste cells (DeSimone et al., 2012, 2015). Furthermore, steady-state changes in taste cell pH_i result from a balance between acid entry across the apical membrane (possibly via Otop1 for strong acids), the sodium–hydrogen exchanger isoform 1 (NHE1)–dependent proton efflux across the basolateral membrane (DeSimone et al., 2015; Tu et al., 2018), and probably other pH-buffering mechanisms in the cell.

Whereas Otop1 is implicated in sour taste transduction and otoconia-dependent vestibular function, the physiological

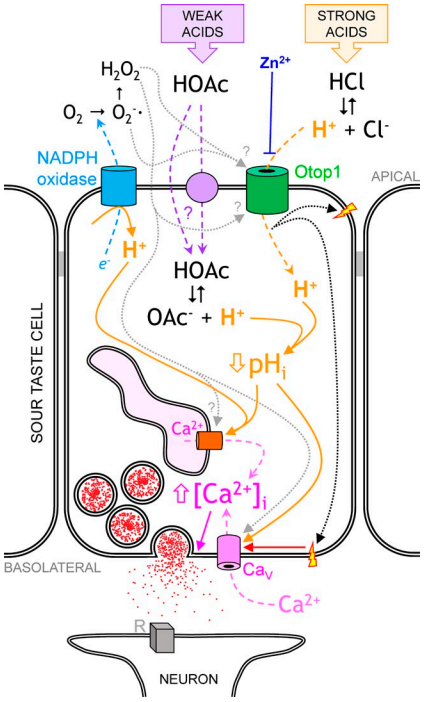

Figure 1. **Sour taste transduction mechanisms for weak and strong acids.** A schematized representation of a sour taste cell containing the newly identified Zn^{2+}-sensitive H^+ channel Otopetrin1 (Otop1, green cylinder), which is proposed to directly mediate entry of strong acid equivalents across the apical membrane. Weak acids (e.g., acetic acid, HOAc) may nonspecifically diffuse across the membrane in their neutral form or be transported by an unindentified pathway (purple circle). Otop1-mediated H^+ currents directly cause membrane depolarization (red lightning bolt), which triggers activation of basolateral voltage-gated Ca^{2+} channels (Ca_V, pink cylinder). Proton entry through the apical H^+ channel and intracellular dissociation of weak acids both result in intracellular acidification ($\downarrow pH_i$), which is linked to a rise in intracellular free Ca^{2+} ($[Ca^{2+}_i]$) by enhanced influx through Ca_V channels and/or release from stores (dashed pink lines). Reactive oxygen species ($O_2^{\cdot-}$ and H_2O_2) produced by NADPH oxidase (blue cylinder) may contribute to pH_i changes elicited by strong and weak acid stimuli by directly altering Otop1 activity or modulating $[Ca^{2+}_i]$, possibly because of effects on Ca_V or intracellular Ca^{2+} store release channels (orange cylinder). Elevating $[Ca^{2+}_i]$ near vesicles (black circles) at the basolateral membrane is thought to increase the probability of neurotransmitter (red stippling) release into the synaptic cleft and activate postsynaptic receptors (R, gray box).

necessity of mammalian Otop2 and Otop3 proteins and the *Drosophila melanogaster* and *Caenorhabditis elegans* otopetrin orthologues is less obvious. Unlike Otop1, current reversal potentials in Otop2 and Otop3 do not strictly follow the Nernst potential for H^+, suggesting that these channels are also permeable to other ions (Tu et al., 2018). In addition, time- and voltage-dependent gating kinetics appear to be more pronounced in Otop1 and Otop3 than in Otop2 (Tu et al., 2018), suggesting differences in gating mechanisms that remain to be elucidated. Furthermore, the linear pH_o dependence of current amplitude in mOtop1, hOtop1, and mOtop3 is distinct from the saturable responses to extracellular acidification in mOtop2 and dmOtopLc (Tu et al., 2018), possibly indicating differences in pH_o and/or pH_i sensing mechanisms among otopetrins. Detailed biophysical studies are needed to elucidate how differences in primary amino acid sequence are related to function. Such data are likely

to prove valuable in helping to decipher the mechanism of H^+ transfer when high-resolution otopetrin structures ultimately become available.

Being the smallest of ions, protons are notoriously difficult to pin down using common biochemical, electrophysiological, and structural methods. The ability of H^+ to migrate in protein and aqueous solution by several biophysically distinct mechanisms confounds efforts to unambiguously identify H^+ transfer pathways in proteins. For example, Grotthuss-type proton transfer (PT) allows H^+ to migrate in single-file chains of hydrogen-bonded water (and ice; DeCoursey, 2003), raising fundamental questions about the mechanism(s) used by otopetrins. Also, net PT can conceivably be accomplished by diffusion of OH^- or H_3O^+ in aqueous-accessible crevices within integral membrane proteins.

We don't yet know enough about biophysical mechanisms in otopetrins to adequately compare them with other H^+-permeable and H^+-selective channels, but now that cDNAs have been identified, some simple predictions can be readily tested. For example, the ability of Zn^{2+} to modulate voltage-dependent gating in the Hv1 proton channel requires two extracellularly accessible His residues (Ramsey et al., 2006), and the pH_o sensitivity of Zn^{2+} block of H^+ currents in taste cells (Bushman et al., 2015) suggests that His side chains could also be involved in Zn^{2+} coordination. However, the larger size and structural complexity, together with a greater number of candidate side chains, are likely to make the identification of residues that mediate Zn^{2+} block in Otop1 an arduous task. Despite knowing the identities of key Zn^{2+}-coordinating residues in Hv1, the effects of Zn^{2+} on Hv1 gating, and the availability of an x-ray structure (Ramsey et al., 2006; Takeshita et al., 2014; Qiu et al., 2016), the mechanism by which divalent metal ions allosterically modulate voltage-dependent gating remains unclear.

Caveat emptor: elucidating the PT mechanism in otopetrins is likely to be a difficult task. Differences in the apparent H^+ selectivity among Otop1–3 suggest that nature may have provided an ideal model system in which to examine the relationship between function and structure. However, the mechanism of PT in WT Hv1, for example, remains hotly debated 12 yr after its identification (Bennett and Ramsey, 2017; DeCoursey, 2017). Although point mutations in Hv1 are reported to erode the normally exquisite H^+ selectivity in Hv1 (Berger and Isacoff, 2011; Musset et al., 2011), our ability to understand the mechanisms that account for mutant effects is severely compromised by the fact that H^+ and OH^- reversal potentials are identical. One hypothesis is compatible with all the available experimental data: both WT and mutant Hv1 proteins accomplish PT using an intraprotein water wire for the activated state "aqueous" H^+ conductance (G_{AQ}; Ramsey et al., 2010). The ability of mutations at a highly conserved Asp (D112/$D^{1.51}$) to readily confer a measurable anion permeability results from removal of the $D^{1.51}$ side chain, which normally prevents permeation of Cl^- through the hydrated central crevice of the Hv1 voltage sensor domain (Randolph et al., 2016; Bennett and Ramsey, 2017). In the absence of the $D^{1.51}$ carboxylate anion, a diffusive anion "leak" current is allowed to flow; nonetheless, hydrogen-bonded waters continue to support unimpeded Grotthuss-type PT between anion permeation events (Randolph et al., 2016; Bennett and Ramsey, 2017). Crucially, the H^+/OH^- selectivity in both WT and mutant Hv1 proteins remains unknown.

The recent identification of otopetrins as new family of H^+-permeable channels (Tu et al., 2018) presents an almost dizzying array of important biophysical, structural, physiological, and behavioral hypotheses to test (and quick). These efforts will advance our understanding of taste transduction, vestibular function, H^+ transfer, and metalloprotein function in fundamentally important ways; other unexpected surprises surely lie in wait. The recent discovery of Tu et al. (2018) would probably not have been possible without continued government support of this important research question. Good early candidates for the sour taste transduction channel (e.g., PKD2L1) were subsequently discarded based on evidence gleaned from carefully executed experiments. The discovery of otopetrins represents a prima facie lesson, this time in physiology and biophysics, that illustrates yet another imperative for unabated government support for basic science (Gad-el-Hak, 2017): experience shows that support of basic science pays dividends to the nation and the global scientific community.

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