Acid-Sensitive Two-Pore Domain Potassium (K\textsubscript{2}P) Channels in Mouse Taste Buds


Acid-sensitive two-pore domain potassium (K\textsubscript{2}P) channels in mouse taste buds. J Neurophysiol 92: 1928–1936, 2004. First published May 12, 2004; 10.1152/jn.00273.2004. Sour (acid) taste is postulated to result from intracellular acidification that modulates one or more acid-sensitive ion channels in taste receptor cells. The identity of such channel(s) remains uncertain. Potassium channels, by regulating the excitability of taste cells, are candidates for acid transducers. Several 2-pore domain K channels, including one or more acid-sensitive ion channels, are candidates for acid transducers. Severa...
TABLE 1.  **RT-PCR primer sequences and GenBank accession numbers for each of the eight K<sub>P</sub> channels examined in the present study**

<table>
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<th>K Channels</th>
<th>Other Names</th>
<th>Gene</th>
<th>Accession Number</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Product size (bp)</th>
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<td>KCNK2</td>
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*Unless otherwise specified, accession numbers are for mouse sequence.

**METHODS**

**Animals**

All experiments were carried out according to the National Institutes of Health Guidelines for the Care and Use of Animals and protocols were approved by the University of Miami Animal Care and Use Committee. Adult C57BL mice (The Jackson Laboratory, Bar Harbor, ME) were killed by exposure to CO<sub>2</sub> followed by cervical dislocation. Tongues were dissected free and transferred to Tyrode's buffer.

**RT-PCR**

Taste epithelium was delaminated by subepithelial injection of proteases as described previously (Gilbertson et al. 1993) and nontaste epithelium surrounding the papilla was trimmed away. The preparation was composed predominantly (but not exclusively) of taste buds. Total RNA was isolated from these taste buds or from adjacent nonsensory lingual epithelium using the Absolutely RNA Nanoprep kit (Stratagene, La Jolla, CA). Taste buds from a single papilla were dispersed in 100 μl lysis buffer containing guanidine thiocyanate and β-mercaptoethanol; RNA from the lysate was captured on a silica-based matrix, treated with DNase I, washed, and then eluted in 10 μl 10 mM Tris-HCl (pH 7.5). Purified RNA was denatured and first-strand cDNA was synthesized at 42°C for 60 min using SuperScript II Reverse Transcriptase in a 20 μl final volume. After removing template RNA with RNase H, 1 μl cDNA was used as template in a 25 μl PCR. Each cDNA preparation was used to test for expression of the entire set of channels described in RESULTS. All reagents were purchased from Invitrogen (Carlsbad, CA).

We designed PCR primers using published full-length cDNA sequences from mouse for TWIK-1 and -2, TREK-1, and TASK-1 and -2 (Table 1). Because no published mouse cDNA sequences were available for TASK-3 or TREK-2, we used full-length cDNAs from rat to identify putative orthologs (>96% identity) in the mouse genome. We confirmed that intron locations and exon sizes were identical between the rat genes and the presumed orthologs in mouse.

For TWIK-1 and -2, TREK-1, and TASK-1 we also amplified product, using primer pairs located in separate exons. Only the forward primer for TASK-1. We also amplified 2 control mRNAs, β-actin (5'-caacgtaaaagatgacc-3', and 5'-ctgaaagAACAg-3', 449 bp product) and the taste cell-specific G protein, gustducin (5'-gaaacctctcagttcatc-3', and 5'-agaagagaAGCgtg-3', 286 bp product), using primer pairs located in separate exons.

**Functional imaging**

We used circumvallate taste buds, which have been shown by us and others to contain acid-responsive taste cells (Caiedo et al. 2002; Richter et al. 2003; Ugawa et al. 2003). We imaged changes of Ca<sup>2+</sup>, pH, and membrane potential in taste cells using one of two preparations: 1) 100-μm-thick slices of circumvallate taste papilla (“lingual epithelium slice”; Richter et al. 2003) or 2) isolated circumvallate taste buds and cells. For imaging, the functional indicator dyes listed in Table 2 were obtained from Molecular Probes (Eugene, OR) and were stored as stock solutions at −20°C.

For Ca<sup>2+</sup> imaging in lingual slices, calcium green dextran (CGD, 1 mM) was injected ionophoretically as described previously (Caiedo and Roper 2001; Caiedo et al. 2002). To record changes in pH in lingual epithelial slices, we loaded taste cells using the same method for the pH-sensitive fluorescent indicator dye, BCECF (Richter et al. 2003a). It should be noted that we measured only relative changes in [Ca<sup>2+</sup>], pH, and membrane potential with this method and were not able to determine the absolute values of [Ca<sup>2+</sup>] and pH at 72°C.

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**Taste stimuli**

Citric acid (100 mM in Tyrode’s buffer, pH ~3), a potent acid taste stimulus in rodents and humans and which, like other weak organic acids...
acids, is a more effective tastant than HCl (e.g., Beatty and Craig 1935), was focally applied with pressure ejection from a puffer micropipette. We measured the concentration of citric acid delivered to the taste cells by including a fluorescent tracer dye in the stimulus solutions (200 μM Lucifer yellow CH; Molecular Probes). For some experiments as indicated, we used a bath application of KCl or acidified Tyrode’s buffer (pH adjusted to 1.5 with HCl).

Microscopy, data analysis, and statistics

Cell responses in the lingual slice preparation were recorded using scanning confocal microscopy with argon laser excitation (488 nm) combined with an FITC filter set (510 LP) to view cells loaded with CGD (Caicedo et al. 2000). Data were captured by scanning a field (~10 μm optical slice) containing 1 or more taste buds every 0.5 to 5.0 s. Data were stored for off-line analysis using Fluoview v. 2.1 (Olympus). Throughout this analysis, we included only data from taste cells that exhibited Ca2+ responses to citric acid with ΔF/F >0.1 and that could be elicited ≥3 times in succession.

When testing the effects of pharmacological agents on taste cell responses (i.e., Δ[Ca2+]i) to citric acid, we included data from cells for which we could collect ≥3 control responses (before drug application) and 3 or more responses during drug treatment. Data from cells that did not recover their control response by 30 min after washout were not included. We used the same selection criteria for ΔpHi, responses of taste cells.

All recordings for individual cells are presented as tracings of ΔF/F. Responses were measured as the peak ΔF/F in a record. Histograms comparing taste cell responses before and after a drug treatment were obtained by taking the mean of 3 or more responses (ΔF/F) before applying a drug and normalizing all responses to that mean. To compare the effects of pharmacological treatments against controls, we used a paired Student’s t-test. For all statistics, P < 0.05 was considered significant. Curves were fitted using Prism (GraphPad Software, San Diego, CA).

Reagents and solutions

Unless stated otherwise, all reagents for physiological experiments were obtained from Sigma (St. Louis, MO). Solutions were prepared freshly for each experiment by dissolving in Tyrode’s buffer (in mM: 135 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, 5 NaHCO3, 10 Hepes, 10 glucose, and 10 sodium pyruvate; pH 7.4). The following pharmacological agents were used at concentrations that are ≥10 times the IC50 for 2-pore domain K+ channels: anandamide (10 μM), Gd3+ (1 mM), arachidonic acid (AA, 100 μM), quinidine (200 μM), quinine (100 mM), and riluzole (500 μM). We also tested the effect of 4-amino-pyridine (4-AP, 10 mM) and tetraethylammonium (TEA, 1 mM) because, although 2-pore domain channels are resistant to these treatments (Lesage 2003), most other potassium channels are not. All test solutions were prepared freshly immediately before experimentation by dissolving in Tyrode’s buffer. Riluzole was prepared as a 100 mM stock solution in DMSO and stored at −80°C. The stock solution was diluted in Tyrode’s buffer to a final concentration of 500 μM (0.1% final DMSO concentration). Halothane was made up as a saturated solution (~17 mM, pH 7.4, cf. Patel et al. 1999). To minimize loss of this volatile anesthetic from the perfusion system, the halothane solution was prepared immediately before experimentation and was delivered by gravity from 10-ml syringes sealed with plastic stoppers.
RESULTS

As we have previously shown (Richter et al. 2003a), applying citric acid focally to the taste pore of vallate taste buds produces an intracellular acidification throughout the entire taste bud (Fig. 1A). In a subset of taste cells, this acidification elicits an increase in \([\text{Ca}^{2+}]_i\) (Fig. 1B).

In electrophysiological studies, acid taste stimulation elicits an inward current and a membrane depolarization in taste receptor cells (Gilbertson et al. 1992; Kinnamon and Roper 1988). We thus postulated that the link between cytoplasmic acidification and the rise in \([\text{Ca}^{2+}]_i\) in acid-responsive taste cells is membrane depolarization followed by activation of voltage-gated Ca channels. Thus we tested whether we could measure depolarizing receptor potentials in acid-sensitive taste cells using the voltage-sensitive fluorescent dye, ANEPPS (Hayashi et al. 1996). We first determined whether changes in ANEPPS fluorescence (\(\Delta F/F_{\text{ANEPPS}}\)) reliably reflected changes in membrane potential in isolated taste buds. To depolarize cells, we applied Tyrode’s buffer with elevated concentrations of KCI (20, 50, and 100 mM; substituted for equimolar NaCl) to isolated taste buds preloaded with ANEPPS. As expected, KCl application resulted in a concentration-dependent increase in \(\Delta F/F_{\text{ANEPPS}}\), consistent with membrane depolarization of taste cells (Fig. 1C). To estimate the incidence of taste cells responding to acid with membrane depolarization, we measured responses from single cells loaded with ANEPPS. Applying citric acid (100 mM) to isolated taste cells resulted in a pronounced increase in ANEPPS fluorescence in about 40% of the isolated cells, consistent with depolarization in a subset of acid-sensitive taste cells (Fig. 1D). Collectively, these findings suggest the model for acid taste transduction shown in Fig. 2.

Expression of acid-sensitive 2-pore domain K channels in mouse taste buds

Using RT-PCR, we asked whether any of the seven K2P channels (Sano et al. 2003), which are modulated by intracellular acidification, were expressed in taste buds and might play a role in acid taste. Expression of five of these acid-sensitive channels was detected in cDNA prepared from mouse circumvallate taste buds, that is, TWIK-1 and -2, TREK-1 and -2, and TASK-1. Expression of TASK-3 and TRESK mRNAs was not apparent (Fig. 3). We found an identical pattern of expression in circumvallate and foliate taste buds for each of these 5 channels (not shown). TASK-1 appeared to be expressed selectively in taste buds when compared with nontaste lingual epithelium. The taste-selective expression pattern of TASK-1 was confirmed in five independent preparations of RNA from mouse circumvallate and foliate taste buds and three preparations from nontaste lingual epithelium. By contrast, RT-PCR products for TWIK-2, TREK-1 and -2, and TASK-2 were readily detected in nontaste lingual epithelium. TWIK-1 appeared to be expressed at a higher level in taste buds compared with nonsensory lingual tissue, although we have not attempted to quantify this differential expression. We also tested for the expression of a related channel, TASK-2, that is activated by external (but not cytoplasmic) acidification. We detected TASK-2 expression at similar levels in taste and nontaste lingual RNAs. PCR for \(\beta\)-actin and the taste cell-specific G protein, \(\alpha\)-gustducin, served to confirm the overall quality and inclusion of taste buds, respectively, in each cDNA preparation.

Although the RT-PCRs in this study were not quantitative, we noted that RT-PCR products corresponding to TWIK-1 and -2 were readily detected after only 30 cycles of amplification, whereas products for the remaining channels required \(\leq 40\) cycles to be consistently detected. This suggests that the corresponding mRNAs for TWIK-1 and -2 may be present at...
relatively high concentrations in taste buds and nonsensory lingual epithelium.

Pharmacological characterization of acid responses

A number of pharmacological agents known to affect the above acid-modulated 2-pore domain K channels were tested for their effect on acid taste responses in mouse taste cells. For example, both quinine and quinidine selectively inhibit TWIK-1 and TASK-2 (Lesage et al. 1996). Continuous perfusion of lingual slices with quinine (200 μM) had no effect on citric acid–induced responses (Fig. 4A). Similarly, 100 mM quinidine did not alter citric acid-induced Ca²⁺ responses (not shown). Parenthetically, other (bitter-sensitive) taste cells did respond to quinine, even at 1 mM, as we have shown previously (Caicedo et al. 2002). The following agents, which act on various 2-pore domain K channels, did not affect citric acid–induced taste responses (Fig. 4A): 10 μM anandamide (an inhibitor of TASK-1; Maingret et al. 2001), 1 mM Gd³⁺ (an inhibitor of TREK-1; Maingret et al. 2000), 100 μM arachidonic acid (an enhancer of TREK-1 and -2; Lesage et al. 2000). We also tested 4-AP and TEA, which are potent inhibitors of voltage-gated and other types of K channels but are relatively ineffective at 2-pore domain K channels (Lesage 2003). Neither 10 mM 4-AP nor 1 mM TEA had an effect on citric acid taste responses (Fig. 4, E and F).

In contrast, we observed that riluzole and halothane consistently altered citric acid-induced Ca²⁺ responses in mouse taste cells. Prolonged application of riluzole depresses TREK-1 and -2 channels (Duprat et al. 2000; Lesage 2003). In the lingual slice preparation, bath-applied, riluzole (500 μM) increased citric acid taste responses by a small, but statistically significant extent (Fig. 5). This effect is consistent with block of a resting (leak) K⁺ conductance.

Halothane, which activates TREK-1 and -2 and TASK-1 and -3 channels (Lesage and Lazdunski 2000; Patel and Honore 2001; Patel et al. 1999; Terrenoire et al. 2001), significantly reduced acid-evoked Ca²⁺ transients in taste cells (P < 0.05, n = 8; Fig. 6, C and D). Nevertheless, halothane did not modify acid-evoked intracellular pH changes in taste cells (P > 0.05, n = 10; Fig. 6, A and B). This result suggests that halothane affects acid taste responses at a step after cytoplasmic acidification but before Ca²⁺ influx. Halothane did not alter responses to a bitter stimulus, cycloheximide (P > 0.05, n = 5, Fig. 6, E and F), demonstrating that this anesthetic does not globally affect taste cell responsiveness.

The above results indicated that halothane perturbs a step downstream of cytoplasmic acidification. Thus we tested whether halothane blocks membrane depolarization in response to acidification, or directly blocks Ca²⁺ influx. To image changes in membrane potential in individual cells, this series of experiments was carried out on isolated taste cells. First, we confirmed that applying halothane did not directly influence either the resting pH or resting Ca²⁺ concentration of taste cells in an isolated preparation (Fig. 7, A and B), just as in the slice. Halothane alone hyperpolarized a significant fraction of ANEPPS-loaded taste cells (Fig. 7C; 31% of cells). This is consistent with the halothane-induced hyperpolarization noted in neurons (Sirotis et al. 1998). Importantly, halothane...
FIG. 6. In the lingual slice preparation, halothane inhibits Ca\textsuperscript{2+} responses elicited by citric acid but cytoplasmic acidification remained unaltered. Taste cells were loaded with BCECF (A, B) or CGD (C–F) and were focally stimulated with citric acid (CA, A) or, as a control, cycloheximide (CX, A). A and B: cytoplasmic acidification produced by focally applied citric acid is not affected by halothane. A: focal stimulation with citric acid elicits decreases in pH throughout the tastebud before (black traces) or during halothane (≥10 mM, gray traces). Six superimposed traces from one taste cell are shown. B: average ΔF/F\textsubscript{BCECF} (± SE, n = 5 cells). Con, control; halo, halothane (≥10 mM). C and D: halothane blocks Ca\textsuperscript{2+} responses elicited by citric acid. C: superimposed traces from a single taste cell focally stimulated with 100 mM citric acid before (black traces) or during halothane (gray traces). D: average Ca\textsuperscript{2+} response to focally applied citric acid before and during halothane (ΔF/F\textsubscript{CGD}, ± SE, n = 4 cells) (*P < 0.05). E and F: halothane does not affect Ca\textsuperscript{2+} responses to a bitter stimulus, cycloheximide. E: superimposed recordings from a taste cell stimulated focally with 100 μM cycloheximide before (black traces) or during halothane (gray traces), as in A–D. F: average response from bitter-sensitive taste cells to focal application of cycloheximide before and during halothane (ΔF/F\textsubscript{CGD}, ± SE, n = 4 cells). Calibrations in A and C are as labeled in E.

also prevented acid-evoked depolarization of isolated taste cells (Fig. 7D). Halothane thus appears to block the Ca\textsuperscript{2+} influx in response to acid stimulation by stabilizing the membrane potential of taste cells.

DISCUSSION

The present study examined a class of potassium leak channels, the K\textsubscript{2P} channels. Specifically, we asked whether members of this group that are sensitive to intracellular acidification are expressed in mouse taste buds and might be involved in sour (acid) taste transduction. We found that TWIK-1 and -2, TREK-1 and -2, and TASK-1, but not TASK-3 or TRESK, channels are expressed in mouse vallate and foliate taste buds. Of these, only TWIK-1 and TASK-1 appeared to be expressed preferentially in taste buds relative to surrounding nontaste epithelium. Preliminary reports have also described TWIK-1, TREK-1 and -2, and TASK-1 and -2 in taste cells from rats (Burks et al. 2003; Lin et al. 2002), although the findings in the rat generally concur with our results in mouse. Burks et al. (2003) reported TASK-3 in vallate and foliate (but not fungiform) taste buds and apparently did not detect TWIK-2. The origin and significance of the differences in expression between rat and mouse taste buds regarding TWIK-2 and TASK-3 expression is unclear. Indeed, there is precedence for such species difference in channel expression. For example, the proton-gated cation channel, ASIC-2, is expressed in rat but not mouse taste buds (Richter et al. 2004).

Several acid-sensitive ion channels have been proposed to mediate sour taste perception in mammals, including the cation channels ENaC, MDEG1, ASIC-2, and HCN1 and HCN4 (Gilbertson and Gilbertson 1994; Gilbertson et al. 1992; Lin et al. 2002; Liu and Simon 2001; Stevens et al. 2001; Ugawa et al. 1998, 2003), and chloride channels (Miyamoto et al. 2000). Downstream of the acid transduction channel(s), a basolateral N\textsuperscript{+}-H\textsuperscript{+} exchanger, NHE-1, is believed to play a significant role in the adaptation of the acid taste response (Lyall et al. 2004). Despite the apparent variety of acid-sensitive ion channels expressed by taste cells, none of the aforementioned ion channels has been directly and unequivocally demonstrated to mediate sour taste. For example, HCN1 and HCN4 are expressed in a subset of taste cells and ionic currents produced by the cloned channel resemble those in some taste cells (Stevens et al. 2001). Similarly, ASIC-2 is expressed in a subset of rat taste cells and ASIC-like currents were recorded in rat taste cells (Ugawa et al. 1998, 2003). In both instances, it is not known whether the cells that express HCN and ASIC2 are those that respond to acid taste stimuli. Further, ASIC-2 is not significantly expressed in mouse taste cells (Richter et al. 2004). Genetic ablation of ASIC-2 in mice does not affect behavioral or Ca\textsuperscript{2+} responses to acid taste stimuli (Kinnamon et al. 2000; Richter et al. 2004). In short, none of the proposed cation or chloride channels has been demonstrated to be a predominantly compelling sour taste transducer in mammalian taste buds.

The absence of an unequivocal sour taste receptor was in part the rationale for investigating a role for K\textsubscript{2P} channels in sour taste. These potassium leak channels are expressed in many tissues. K\textsubscript{2P} channels are constitutively active and play a major role in physiological functions including establishing membrane potential and regulating neuronal and muscular excitability in response to neurotransmitters and hormones (Lesage and Lazdunski 2000). Some members of the K\textsubscript{2P} family are regulated by pH changes in the extracellular milieu or in the cytoplasm or both, whereas others are relatively pH insensitive. We have tested those that are affected by intracellular acidification, which is believed to be the proximate stimulus for acid taste (Lyall et al. 2001). One such channel, TASK-1, is a proposed acid sensor in the carotid body (Buckler et al. 2000; Richter et al. 2004). Genetic ablation of ASIC-2 in mice does not affect behavioral or Ca\textsuperscript{2+} responses to acid taste stimuli (Kinnamon et al. 2000; Richter et al. 2004). In short, none of the proposed cation or chloride channels has been demonstrated to be a predominantly compelling sour taste transducer in mammalian taste buds.
et al. 2000). It may be significant that we found TASK-1 is expressed in taste buds and not in surrounding nonsensory tissue. TASK-1 is blocked by both intra- and extracellular acidification (Kim et al. 1999). Thus acid taste stimulation should depolarize taste cells that express this channel. Indeed, citric acid stimulation was shown to result in depolarizing receptor potentials that originate from a blocked resting conductance (Cummings and Kinnamon 1992). In short, TASK-1 might be a good candidate for an acid taste transducer in mouse taste buds. Opposing this conclusion, however, is the lack of action of anandamide (a TASK-1 blocker) on acid taste responses in our experiments (Fig. 4B). Further studies using in situ hybridization, immunohistochemistry, and more detailed functional analyses would be necessary to definitively establish or refute a role for TASK-1 in sour taste.

The results from the pharmacological testing did not allow us to identify unambiguously any one acid-sensitive K_2P channel as uniquely associated with acid taste responses. Expression alone would suggest TWIK-1 and TASK-1 are the most relevant channels because they are preferentially expressed in taste cells. However, this conclusion was not supported by the pharmacological profile of acid responses in our experiments.

The effects of riluzole on acid responses are consistent with an involvement of TREK-1 and -2, both of which are blocked by riluzole. However, because intracellular acidification opens TREK-1 and -2 channels to hyperpolarize cells (Lesage and Lazdunski 2000), neither of these channels is a candidate for a primary transducer for acid taste. One would anticipate that pharmacological block of TREK-1 and -2 leak conductances would enhance any acid-evoked depolarizing currents, in line with what we observed for taste cells (Fig. 5). This would suggest that TREK-1 and/or TREK-2 might act as modulatory channels in acid-sensing taste cells, serving to oppose acid-evoked responses and thereby keeping depolarizing receptor potentials in check, or assisting in recovery from depolarization. A major caveat is that, although riluzole has been widely used to diagnose K_2P channels in numerous studies, it has been shown to affect other ion channels as well (Cao et al. 2002). Similarly, the ability of halothane to depress acid taste responses may result from its known ability to activate TREK-1 and TASK-1 channels. By opening these leak conductances, halothane hyperpolarizes membranes and shunts inward currents, thus preventing depolarizing receptor potentials. These outcomes readily explain the results in taste cells (Fig. 7, C and D). Yet, as with riluzole, the actions of halothane are not completely specific to K_2P channels. For instance, halothane also blocks voltage-gated Ca channels (Kamatchi et al. 1999), an action that would cooperate with its hyperpolarizing effect on membrane potential to further reduce any acid-evoked Ca\(^{2+}\) influx (Fig. 6, C and D).

In aggregate, our findings are generally consistent with—but do not prove—a role for K_2P channels in taste in general, and acid taste in particular. Certain of the acid-sensitive K_2P channels may be more likely than others to be involved in sour taste because of their preferential expression in taste buds versus nontaste tissue (i.e., TWIK-1 and TASK-1) and because
rutilzole and halothane affected acid taste responses (i.e., TREK-1 and -2 and TASK-1). It is possible that acid taste transduction is the result of the effect of intracellular acidification on a matrix of acid-sensitive ion channels in taste cells, some of which would tend to depolarize (e.g., ASIC2, HCN-1 and -4, TASK-1) and others, like TREK-1 and -2 channels, to stabilize the membrane potential, with a net depolarizing receptor potential. If this interpretation is correct, it may be difficult to use knockout mice to isolate any one contributor to sour taste.

As a footnote, throughout this report the assumption has been that intracellular acidification in the taste bud is the proximal stimulus for acid taste responses, as proposed by others (Lyall et al. 2001) and as we have also observed (Richter et al. 2003a). Yet it should be noted that applying acid taste stimuli to the lingual sensory surface will acidify intra- and extracellular compartments alike. Measurements of pH alterations selectively in the narrow intercellular spaces within taste buds after acid taste stimulation have not been possible to date. This may be important because extracellular, rather than intracellular, protons affect different K,iP leak channels. It might also be noted that ASIC-2, a candidate sour taste transducer in rat taste cells (Ugawa et al. 1998, 2003) is gated by extracellular protons. Further, ENaC channels would require a proton gradient across the membrane (i.e., extracellular > intracellular concentration) to generate inward current carried by H+. Ambiguity about the actual site of action of protons released from sour tastants only adds to the present uncertainties about sour taste transduction.

ACKNOWLEDGMENTS

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GRANTS

This study was supported by National Institute on Deafness and Other Communication Disorders Grants 2R01 DC-00374 to S. D. Roper and 1R01 DK-63516 to Buckler KJ, Williams BA, and Honore E. This study was supported by National Institute on Deafness and Other Communication Disorders Grants 2R01 DC-00374 to S. D. Roper and 1R01 DK-63516 to Buckler KJ, Williams BA, and Honore E.

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