

# Decrease in rat taste receptor cell intracellular pH is the proximate stimulus in sour taste transduction

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<sup>1</sup>Department of Physiology, Virginia Commonwealth University, Richmond 23298-0551, <sup>3</sup>McGuire Veterans Affairs Medical Center, Richmond, Virginia 23249; and <sup>2</sup>Department of Physiology and Biophysics, Indiana University School of Medicine, Indianapolis, Indiana 46202-5120

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**Lyall, Vijay, Rammy I. Alam, Duy Q. Phan, Glenn L. Ereso, Tam-Hao T. Phan, Shahbaz A. Malik, Marshall H. Montrose, Shaoyou Chu, Gerard L. Heck, George M. Feldman, and John A. DeSimone.** Decrease in rat taste receptor cell intracellular pH is the proximate stimulus in sour taste transduction. *Am J Physiol Cell Physiol* 281: C1005–C1013, 2001.—Taste receptor cells (TRCs) respond to acid stimulation, initiating perception of sour taste. Paradoxically, the pH of weak acidic stimuli correlates poorly with the perception of their sourness. A fundamental issue surrounding sour taste reception is the identity of the sour stimulus. We tested the hypothesis that acids induce sour taste perception by penetrating plasma membranes as H<sup>+</sup> ions or as undissociated molecules and decreasing the intracellular pH (pH<sub>i</sub>) of TRCs. Our data suggest that taste nerve responses to weak acids (acetic acid and CO<sub>2</sub>) are independent of stimulus pH but strongly correlate with the intracellular acidification of polarized TRCs. Taste nerve responses to CO<sub>2</sub> were voltage sensitive and were blocked with MK-417, a specific blocker of carbonic anhydrase. Strong acids (HCl) decrease pH<sub>i</sub> in a subset of TRCs that contain a pathway for H<sup>+</sup> entry. Both the apical membrane and the paracellular shunt pathway restrict H<sup>+</sup> entry such that a large decrease in apical pH is translated into a relatively small change in TRC pH<sub>i</sub> within the physiological range. We conclude that a decrease in TRC pH<sub>i</sub> is the proximate stimulus in rat sour taste transduction.

hydrogen ion conductance; intracellular signaling; chorda tympani; acid stimulation

SOURNESS is a primary taste sensation (25). Stimuli evoking a sour sensation yield dissociable H<sup>+</sup> ions. It is natural to assume that taste receptor cells (TRCs) are extracellular pH (pH<sub>o</sub>) detectors, and that sourness should, therefore, be a graded function of stimulus pH. However, this is not generally true. The poor correlation between sourness and stimulus pH has been amply demonstrated in both human (1, 12, 17, 21, 24, 29) and animal (2, 3, 27) studies. At the same pH, acetic acid is a more potent sour stimulus than HCl (12, 27, 29). Moreover, the sourness of acetic acid (and other

weak acids) is essentially the same as that of a buffer consisting of the acid plus its conjugate base, even though the latter has a higher pH (2, 12). It has been speculated that weak acids might induce sour taste perception by penetrating plasma membranes as undissociated molecules and decreasing intracellular pH (pH<sub>i</sub>) upon dissociating within the TRCs (13, 27, 35). We have previously demonstrated that isolated rat (23) and hamster (34) TRCs respond to changes in pH<sub>o</sub> with parallel changes in pH<sub>i</sub>. This suggests that changes in TRC pH<sub>i</sub> may indeed be the appropriate stimulus variable for sour taste transduction. If so, it would explain the paradoxical correlation between stimulus pH and sourness. However, before a firm conclusion can be drawn regarding the role of pH<sub>i</sub> in acid sensing, measurements of TRC pH<sub>i</sub> must be made in an intact taste bud maintained with its natural polarity, and a direct correlation must be established between changes in pH<sub>i</sub> of polarized TRCs and the taste nerve responses to acids. Finally, pharmacological agents that specifically inhibit the normal acid-induced decrease in pH<sub>i</sub> should also inhibit the acid-induced neural response. We have made these measurements, and the results demonstrate unequivocally that changes in pH<sub>i</sub> in TRCs represent the proximate sour taste stimulus in rat.

## MATERIALS AND METHODS

**Chorda tympani recording.** Female Sprague-Dawley rats (150–200 g) were anesthetized by intraperitoneal injection of pentobarbital sodium (60 mg/kg), and supplemental pentobarbital (60 mg/kg) was administered as necessary to maintain surgical anesthesia. Body temperatures were maintained at 36–37°C with a circulating-water heating pad. The left chorda tympani (CT) nerve was exposed laterally as it exited the tympanic bulla (9, 34, 39) and placed onto a 32-gauge platinum-iridium wire electrode. An indifferent electrode was placed in nearby tissue. Neural responses were differentially amplified with a custom-built, optically coupled isolation amplifier. For display, responses were filtered with the use of a band-pass filter with cut-off frequencies of 40 Hz–3 kHz and fed to an oscilloscope. Responses were then

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full-wave rectified and integrated with a time constant of 1 s. Integrated neural responses and current and voltage records were recorded on a Linseis TYP7045 chart recorder and also captured on disk with Labview software and were analyzed off-line (40). Stimulus solutions were injected into a Lucite chamber (3 ml; 1 ml/s) affixed by vacuum to a 28-mm<sup>2</sup> patch of anterior dorsal lingual surface. The chamber was fitted with separate Ag-AgCl electrodes for measurement of current and potential. These electrodes served as inputs to a voltage-current clamp amplifier that permitted the recording of neural responses with the chemically stimulated receptive field under current or voltage clamp (39, 40). The clamp voltages were referenced to the mucosal side of the tongue. The anterior lingual surface was stimulated with a rinse solution (10 mM KCl) and with acetic acid, citric acid, or HCl solutions at pH 3.0 or with solutions containing 10 mM of each of the above acids. To measure the CT response to dissolved CO<sub>2</sub>, we stimulated the lingual surface with a solution containing 72 mM KHCO<sub>3</sub> buffered to pH 7.4 with a 10% CO<sub>2</sub>-90% O<sub>2</sub> mixture. In these experiments the rinse solutions contained 72 mM KCl buffered to pH 7.4 with 10 mM HEPES. To evaluate the role of carbonic anhydrase in dissolved-CO<sub>2</sub>-induced CT responses, we studied the effect of topical application of 50 mM MK-417 (Merck, Rahway, NJ), a cell-permeable blocker of carbonic anhydrase (11), on CT responses. In some experiments the CT responses were recorded at the slower perfusion rate of 1 ml/min to obtain flow rates comparable to those required in pH<sub>i</sub> measurements in isolated single fungiform papillae.

**pH<sub>i</sub> measurements.** Rats were anesthetized with methoxyflurane and killed by cervical dislocation. The tongues were rapidly removed and stored in ice-cold Ringer solution, containing (in mM) 140 NaCl, 5 KCl, 1 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 10 Na-pyruvate, 10 glucose, and 10 HEPES, pH 7.4. The lingual epithelium was isolated by collagenase treatment (23, 34). A small piece of the anterior lingual epithelium containing a single fungiform papilla (Fig. 1, *a-d*) was mounted in a special microscopy chamber (7). The tissue was intermittently perfused with Ringer solution containing 25 μM of the pH-sensitive fluoroprobe 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein-AM (Molecular Probes, Eugene, OR) at 4°C for 2 h. Before the experiment was started, the tissue was perfused on both sides with control solution for 15 min. The control solution was Ringer solution without Na-pyruvate. The tissue was perfused at the rate of 1 ml/min. The TRCs in

the taste bud were visualized from the basolateral side through a ×40 objective (Zeiss; 0.9 NA) with a Zeiss Axioskop microscope and imaged with a setup consisting of a cooled charge-coupled device camera (Imago, TILL Photonics Applied Scientific Instrumentation, Eugene, OR) attached to an image intensifier (model VS4-1845; Videoscope, Washington, DC), an epifluorescent light source (TILL Photonics Polychrome IV), a 515-nm dichroic beam splitter (Omega Optical), and a 535-nm emission filter (20-nm band pass; Omega Optical). The cells were alternately excited at 490 and 440 nm and imaged at 10-s intervals. Small regions of interest (ROIs) in the taste bud (diameter 2–3 μm<sup>2</sup>) were chosen in which the changes in fluorescence intensity ratio (F<sub>490</sub>/F<sub>440</sub>) were analyzed using TILLvisION v3.1 imaging software. The background and autofluorescence at 490 and 440 nm were corrected from images of a taste bud without the dye. The changes in TRC pH<sub>i</sub> were calibrated by bilateral perfusion of high-K<sup>+</sup> calibrating solutions between pH 6.5 and 8.0 containing 10 μM nigericin (23, 34). All experiments were performed at room temperature (22 ± 1°C).

**Stimulus solutions.** Stimulus solutions were without HEPES and contained HCl, acetic acid, citric acid, and tartaric acid at the concentrations given in the text. Stimulus solution containing 8.9 mM acetic acid (pH 3.2) was titrated to pH 6.0 with 155 mM potassium acetate (155 mM potassium acetate replaced 150 mM NaCl and 5 mM KCl in the solution). Bicarbonate-buffered solution contained (in mM) 78 NaCl, 5 KCl, 1 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 10 glucose, and 72 NaHCO<sub>3</sub> and was bubbled with a 10% CO<sub>2</sub>-90% O<sub>2</sub> mixture (pH 7.4). In some experiments both HEPES- and bicarbonate-buffered solutions contained either 5 or 50 mM MK-417, a membrane-permeable blocker of carbonic anhydrases (11).

**Data analysis.** Results are presented as means ± SE of the number of ROIs in the taste bud. Student's *t*-test was employed to analyze the differences between sets of data.

## RESULTS

**Effect of acid stimulation on CT nerve activity.** The lingual application of acid stimuli increases CT nerve activity (Fig. 2). At pH 3.0 (Fig. 2A), acetic acid gave a larger CT response than either citric acid or HCl, indicating that acetic acid is a stronger sour taste stimulus than citric acid and HCl (2, 12, 24, 27). In contrast, when each acid was applied at a concentra-

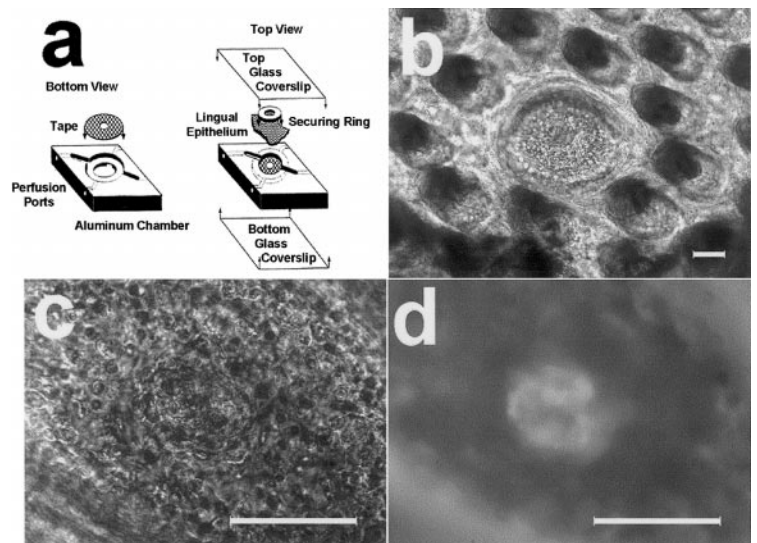


Fig. 1. Microscopy chamber. *a*: mounting of an isolated piece of lingual epithelium (7) containing a single fungiform papilla in the microscopy chamber. Images (*b-d*) represent transmitted and fluorescent images of the taste bud when viewed from the basolateral side (bar, 10 μm) at ×10 and ×40 magnification. Individual taste receptor cells (TRCs) can be observed in the taste bud region (*c*). The fluorescence image of the same taste bud excited at 490 nm is shown (*d*). The dye is specifically loaded in TRCs and is excluded from the surrounding supporting cells.

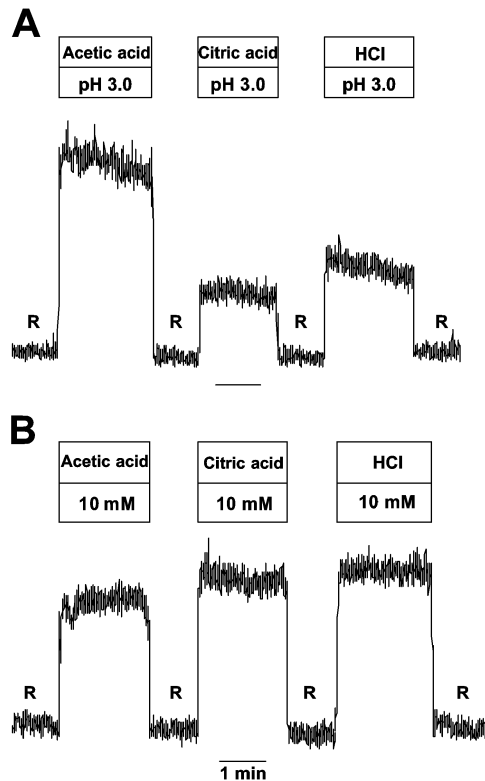


Fig. 2. Effect of acid stimuli on left chorda tympani (CT) nerve activity. The integrated CT responses were monitored in anesthetized rats while the anterior lingual surface was suffused with a rinse solution (R; 10 mM KCl) and with solutions containing acetic acid, citric acid, or hydrochloric acid (HCl) at pH 3.0 (A) or with solutions containing 10 mM acids (B).

tion of 10 mM, all three acids produced CT responses of similar magnitude even though the pH values of the acid solutions varied between 2.0 and 3.4 (Fig. 2B). Thus CT responses are not graded functions of the lingual surface pH ( $pH_o$ ) (2, 12, 24, 27).

**Effect of acid stimulation on TRC  $pH_i$ .** Acidic stimuli were applied to the apical side while changes were monitored in TRC  $pH_i$  in situ from the basolateral side. Acidic stimuli decreased TRC  $pH_i$  (Fig. 3, A–C). Acetic acid at pH 3.1 induced greater decreases in TRC  $pH_i$  than citric acid, tartaric acid (Fig. 3A), and HCl (Fig. 3B). In contrast, the changes in  $pH_i$  produced by individual acids at 10 mM concentration were of similar magnitude (Fig. 3C). In each case, therefore, the decrease in  $pH_i$  correlates with the respective taste neural responses, while  $pH_o$  does not.

At pH 3.0, the acetic acid concentration is 58.3 mM. Of this, 57.3 mM is undissociated acid. Citric acid, a much stronger acid, has a concentration of 2.2 mM, of which 1.19 mM is undissociated. The corresponding parameters for tartaric acid are similar to those for citric acid. At pH 3.0, acetic acid induced a greater decrease in TRC  $pH_i$  (Fig. 3A) and a greater CT response (Fig. 2A) for two reasons: 1) it is present at higher concentration than either citric acid or tartaric acid, and 2) a much higher proportion of the acetic acid is present as the membrane-permeable undissociated

form (13). At 10 mM concentration, acetic acid has a pH of 3.4. Of this, 9.59 mM is undissociated, while 10 mM citric acid has a pH of 2.6 and 7.49 mM is undissociated. The concentrations of the undissociated forms of acetic acid and citric acid in the stimulus solutions are comparable and, therefore, induce comparable changes in  $pH_i$  (Fig. 3C) and CT responses (Fig. 2B) for both

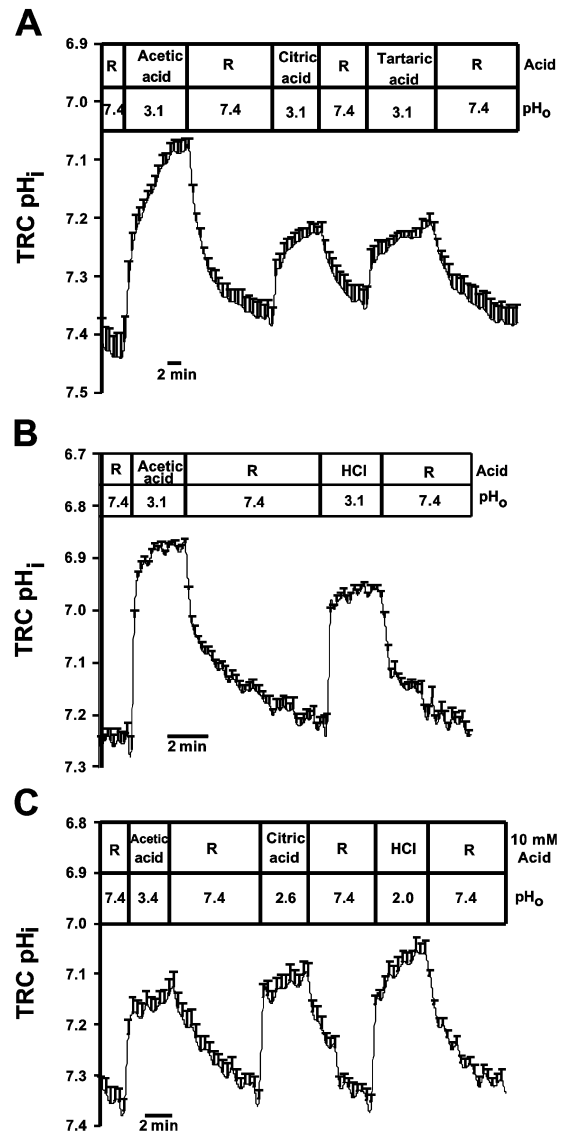


Fig. 3. Acid-induced decrease in intracellular pH ( $pH_i$ ) of polarized TRCs. TRC  $pH_i$  was monitored while the tissue was perfused on both sides with control solution (R; pH 7.4) and during perfusion of the lingual surface with solutions containing acids at pH 3.1 (A and B) or acids at 10 mM concentration (C). A: a decrease in extracellular pH ( $pH_o$ ) from 7.4 to 3.1 ( $\Delta pH_o = 4.3$ ) with acetic acid, citric acid, and tartaric acid decreased the mean  $pH_i$  in a taste bud by 0.34, 0.16 and 0.13, respectively [ $n = 3$  regions of interest (ROIs)]. The ratio  $\Delta pH_i / \Delta pH_o$  for these 3 acids was 0.08, 0.04, and 0.03, respectively. B: in another taste bud, acetic acid and HCl at pH 3.1 decreased mean  $pH_i$  by 0.37 and 0.27, respectively ( $n = 5$  ROIs). The  $\Delta pH_i / \Delta pH_o$  values for these 2 acids were 0.086 and 0.063, respectively. C: when the acids were all present at 10 mM concentration, the mean change in  $pH_i$  for acetic acid, citric acid, and HCl was 0.23, 0.21, and 0.24, respectively ( $n = 4$  ROIs). Similar results were obtained in 2 other experiments.

acids. Within the normal range of  $pH_i$  (Fig. 3, A and C), diffusing citric acid delivers three equivalents of  $H^+$  ions to the TRCs. This suggests that the intrinsic permeability of acetic acid is about three times that of citric acid.

**Effect of  $pH_o$  on acetic acid responses.** If the permeability of the undissociated form of a weak acid is the critical factor in determining its intensity as a stimulus, the changes in  $pH_i$  and the CT response should both be independent of  $pH_o$ . To test this hypothesis, we obtained the CT responses to unbuffered acetic acid ( $pH \sim 3$ ) and to the same concentration of acetic acid buffered at pH 6 with potassium acetate. At a given concentration, the buffered (pH 6.0) and unbuffered acetic acid (pH 3.0) gave similar responses (Fig. 4A). In parallel experiments, perfusing solutions containing 8.9 mM acetic acid plus 150 mM potassium acetate (pH 5.9) and 8.9 mM acetic acid alone (pH 3.2) across the apical side of a single fungiform papilla induced changes in TRC  $pH_i$  of similar magnitude (Fig. 4B). Thus the CT response and the changes in TRC  $pH_i$  are independent of  $pH_o$  and the acetate concentration. Both the CT response and the changes in TRC  $pH_i$  were found to be independent of apical  $Na^+$  and were unaffected by 1 mM  $\alpha$ -cyano-4-hydroxycinnamate (4), a blocker of monocarboxylate transporter I (unpublished observations). The data suggest that undissociated acetic acid molecules enter TRCs by passive diffusion across the apical membranes (13).

**Effect of  $CO_2$  on CT nerve activity.** Initially the lingual surface was suffused with a rinse solution containing 72 mM KCl buffered to pH 7.4 with 10 mM HEPES, and the CT activity was taken as the baseline activity. Suffusing the lingual surface with a solution containing 72 mM  $KHCO_3$  buffered to pH 7.4 with 10%  $CO_2$ -90%  $O_2$  produced reversible increases in CT activity (Fig. 5A). Cell membranes are freely permeable to dissolved  $CO_2$ , and its conversion to  $H_2CO_3$  catalyzed by intracellular carbonic anhydrase (8, 16) represents penetration of acid equivalents across TRC membranes (23, 34). This was tested directly by recording CT responses in the presence of MK-417, a specific blocker of carbonic anhydrases (11). We applied 50 mM MK-417 topically on the lingual surface in the rinse solution for 15 min. This inhibited the dissolved- $CO_2$ -induced CT responses by  $\sim 50\%$  (Fig. 5A, middle). Upon washout, the effects of MK-417 were completely reversible (Fig. 5A, right). In a similar study (20), topical application of MK-927, another membrane-permeable carbonic anhydrase inhibitor, to the rat tongue inhibited CT responses to carbonated water by 62%.

**Voltage dependence of  $CO_2$  induced CT nerve activity.** Data summarized in Fig. 5B indicate that unlike CT responses to HCl (9, 34) and acetic acid (data not shown), the CT responses induced by dissolved  $CO_2$  are voltage sensitive. Compared with open-circuit conditions (i.e., under 0 current clamp), the CT responses were enhanced at  $-60$  mV and suppressed at  $+60$  mV, similar to voltage-clamp effects on responses to NaCl (39, 40).

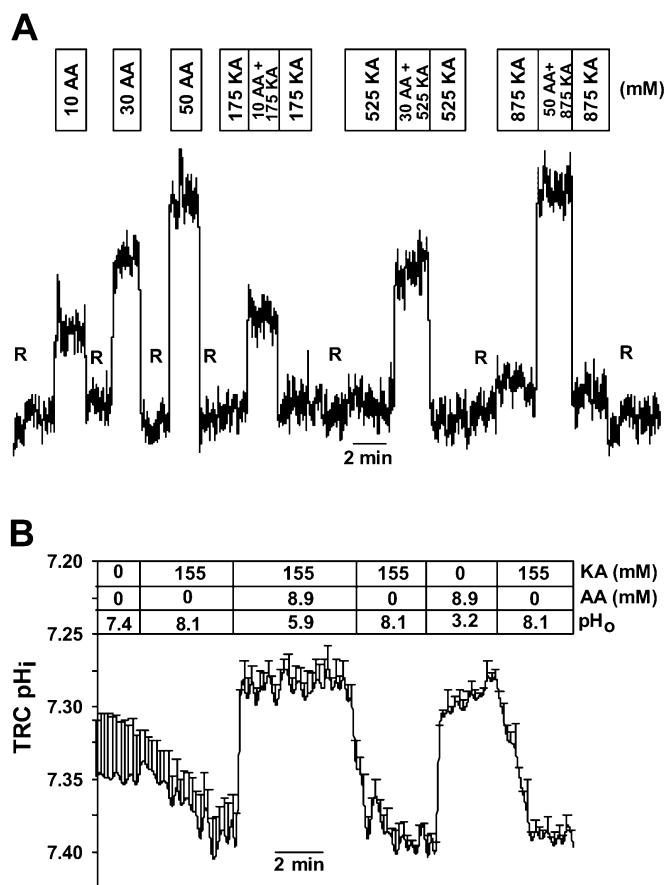


Fig. 4. Effect of  $pH_o$  on acetic acid-induced changes in CT responses and TRC  $pH_i$ . **A:** CT responses were recorded while the lingual surface was perfused with solutions containing 10 (pH 3.40), 30 (pH 3.15), and 50 mM (pH 3.05) acetic acid (AA) or with solutions containing 10, 30, and 50 mM acetic acid titrated to pH 6.0 with 175, 525, and 875 mM potassium acetate (KA), respectively. The CT responses increased with acetic acid concentration and were independent of  $pH_o$ . The solutions containing 175 and 525 mM potassium acetate (pH  $> 9.0$ ) alone did not produce significant changes in CT activity. At 875 mM potassium acetate, a small CT response was noted. Similar results were obtained in 2 other experiments. **B:** TRC  $pH_i$  was monitored while the tissue was perfused on both sides with control solution (pH 7.4) and when the lingual surface was perfused with an unbuffered solution containing 155 mM potassium acetate (with KA replacing all NaCl and KCl in the Ringer solution) at pH 8.1. The mean TRC  $pH_i$  in the presence of 155 mM potassium acetate solution was taken as the baseline  $pH_i$ . In the second step, perfusing a solution of 155 mM potassium acetate buffered to pH 5.9 with 8.9 mM acetic acid produced a reversible decrease in TRC  $pH_i$  from baseline. In the third step, perfusing a solution containing 8.9 mM acetic acid alone (pH 3.2) produced a decrease in TRC  $pH_i$  similar to that observed in the presence of 155 mM potassium acetate plus 8.9 mM acetic acid (pH 5.9). Values are means  $\pm$  SE of 3 ROIs in the taste bud.

**Effect of  $CO_2$  on TRC  $pH_i$ .** The generation of intracellular  $H^+$  ion was confirmed directly by the observed decrease in TRC  $pH_i$  produced by perfusing the lingual surface with a solution containing 72 mM  $NaHCO_3$  buffered to pH 7.4 with 10%  $CO_2$ -90%  $O_2$  (Fig. 6A). The changes in TRC  $pH_i$  were significantly attenuated in the presence of 5 mM MK-417 (Fig. 6B). In the presence of 50 mM MK-417, dissolved  $CO_2$  induced a maximum decrease in  $pH_i$  of  $0.05 \pm 0.008$  pH unit. These

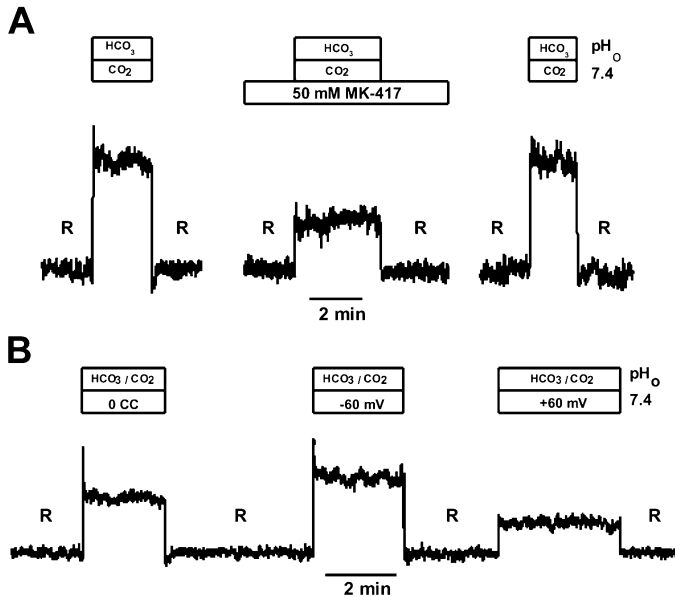


Fig. 5.  $\text{CO}_2$ -induced changes in CT activity. *A*: CT responses were recorded while the lingual surface was suffused with rinse solution ( $R = 72 \text{ mM KCl}$  solution buffered to  $\text{pH } 7.4$  with HEPES) or with  $72 \text{ mM KHCO}_3$  solution buffered to  $\text{pH } 7.4$  with a  $10\% \text{ CO}_2$ - $90\% \text{ O}_2$  gas mixture (*left*). The lingual surface was treated with rinse solution containing  $50 \text{ mM MK-417}$  for  $15 \text{ min}$ , and the CT responses were recorded (*middle*). The lingual surface was then rinsed with the rinse solution without the drug for  $15 \text{ min}$ , and CT responses were recorded again (*right*). *B*: responses to  $72 \text{ mM KHCO}_3$  solution buffered to  $\text{pH } 7.4$  with a  $10\% \text{ CO}_2$ - $90\% \text{ O}_2$  gas mixture were recorded under open-circuit conditions [i.e., under zero current clamp ( $0 \text{ CC}$ )], at  $-60 \text{ mV}$ , and at  $+60 \text{ mV}$  of voltage clamp.

data suggest a direct role of carbonic anhydrase in  $\text{CO}_2$ -induced sour taste transduction (20). Dissolved  $\text{CO}_2$  entry across the apical membrane of TRCs is independent of  $\text{pH}_o$  and the  $\text{HCO}_3^-$  concentration in the stimulus solution. The fact that this acidic stimulus can be presented at an alkaline  $\text{pH}_o$  is further evidence that the proximate acidic stimulus is the change in  $\text{pH}_i$  and not  $\text{pH}_o$ . Fully dissociated strong acids, such as  $\text{HCl}$  (Fig. 1),  $\text{HNO}_3$ , and  $\text{H}_2\text{SO}_4$ , are also potent stimuli of the taste nerves (2, 3, 9, 24, 30) and decrease TRC  $\text{pH}_i$  (Fig. 3, *B* and *C*), suggesting that  $\text{H}^+$  ions also gain entry into TRCs but, in all probability, not by diffusing through the membrane lipid bilayer like dissolved  $\text{CO}_2$  and the neutral forms of weak acids.

*Temporal relation between CT activity and TRC  $\text{pH}_i$ .* Consistent with our previous studies (9, 34, 39, 40), CT nerve activity was monitored in vivo at normal physiological temperature while the lingual surface was suffused with stimulating solutions at the rate of  $1 \text{ ml/s}$ . In contrast, our in vitro  $\text{pH}_i$  measurements were made in a small microscopy chamber at room temperature where the maximum rates of perfusion were  $1 \text{ ml/min}$ . It is desirable to control for these differences in the rate of stimulus application when comparing the CT recordings with the changes in TRC  $\text{pH}_i$ . It was not possible, for technical reasons, to increase the flow rate in vitro beyond  $1 \text{ ml/min}$ . Therefore, we made some CT recordings with stimulus and rinse applied at  $1 \text{ ml/min}$ . Consistent with previous studies (10, 31), the

phasic parts of the CT responses were strongly influenced by the flow rate (Fig. 7); however, the magnitude of the maximum CT response to either dissolved  $\text{CO}_2$  (Fig. 7*A*) or  $\text{HCl}$  at  $\text{pH } 3.0$  (Fig. 7*B*) was not affected by the flow rate. At the flow rate of  $1 \text{ ml/min}$  both acid stimuli, dissolved  $\text{CO}_2$  (Fig. 8, *A* and *B*) and  $\text{HCl}$  (Fig. 8, *C* and *D*) induced CT-response profiles that were similar to the  $\text{pH}_i$  changes observed in vitro. The data show that under the additional constraint of comparable flow rates, the CT nerve response and the changes in TRC  $\text{pH}_i$  are temporally well correlated.

*Regional differences in HCl- and acetic acid-induced changes in pH within a taste bud.* No CT responses were observed with  $\text{HCl}$  at  $\text{pH } 5.0$  (data not shown). A decrease in  $\text{pH}_o$  from  $7.4$  to  $5.0$  induced an average decrease in TRC  $\text{pH}_i$  of  $0.11 \pm 0.003 \text{ pH unit}$  in a taste bud ( $n = 12$ ). We compared the regional changes in TRC  $\text{pH}_i$  in three taste buds exposed to apical acetic acid or  $\text{HCl}$  at  $\text{pH } 3.0$ . All 58 ROIs in taste buds responded with a decrease in  $\text{pH}_i > 0.11 \text{ pH unit}$ . The mean decrease in  $\text{pH}_i$  produced by acetic acid ( $\Delta\text{pH}_i = 0.40 \pm 0.01$ ) was significantly greater than that pro-

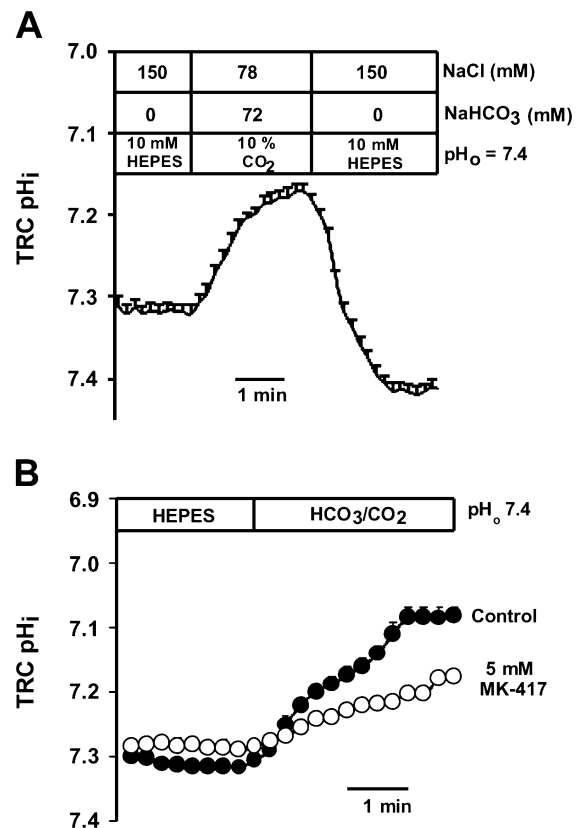


Fig. 6.  $\text{CO}_2$ -induced changes in TRC  $\text{pH}_i$ . *A*: TRC  $\text{pH}_i$  was monitored while the tissue was perfused on both sides with control solution buffered to  $\text{pH } 7.4$  with HEPES and when the lingual surface was perfused with the solutions buffered to  $\text{pH } 7.4$  with  $72 \text{ mM NaHCO}_3$  plus  $10\% \text{ CO}_2$ - $90\% \text{ O}_2$  ( $72 \text{ mM NaHCO}_3$  replaced  $72 \text{ mM NaCl}$  in the solution). *B*: TRC  $\text{pH}_i$  was monitored while the tissue was perfused on both sides with control solution (HEPES) and when the lingual surface was perfused with the solutions buffered to  $\text{pH } 7.4$  with  $72 \text{ mM NaHCO}_3$  plus  $10\% \text{ CO}_2$ - $90\% \text{ O}_2$  ( $\text{HCO}_3^-/\text{CO}_2$ ) in the presence ( $\circ$ ) and absence ( $\bullet$ ) of  $5 \text{ mM MK-417}$ . Values are means  $\pm$  SE of 7 ROIs in the taste bud.

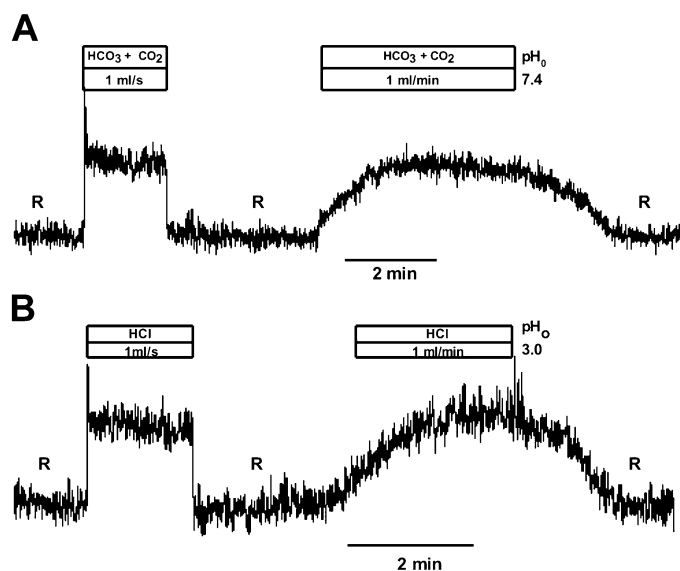


Fig. 7. Effect of flow rate on CT responses. *A*: CT responses were recorded while the lingual surface was suffused with rinse solution ( $R = 72$  mM KCl solution buffered to pH 7.4 with HEPES) or with 72 mM  $\text{KHCO}_3$  solution buffered to pH 7.4 with a 10%  $\text{CO}_2$ -90%  $\text{O}_2$  gas mixture at the rate of 1 ml/s or 1 ml/min. *B*: CT responses were recorded while the lingual surface was suffused with rinse solution ( $R = 10$  mM KCl) or with HCl solution at pH 3.0 at the rate of 1 ml/s or 1 ml/min.

duced by HCl ( $\Delta\text{pH}_i = 0.29 \pm 0.01$ ;  $P < 0.001$ ; paired). In 56 ROIs, acetic acid produced a greater decrease in  $\text{pH}_i$  than HCl did. The changes in  $\text{pH}_i$  varied widely within ROIs. After HCl treatment (Fig. 9A), 35 of 58 ROIs responded with a decrease in  $\text{pH}_i$  between 0.2 and 0.299 pH unit, and 20 of 58 ROIs (34.5%) responded with a decrease in  $\text{pH}_i > 0.3$  pH unit. It is likely that TRCs in the ROIs that respond with the greatest decrease in  $\text{pH}_i$  participate most in sour transduction. In contrast, after acetic acid treatment, 5 of 58 ROIs responded with a decrease in  $\text{pH}_i$  between 0.2 and 0.299 pH unit, and 53 of 58 ROIs (91.4%) changed  $\text{pH}_i > 0.3$  pH unit (Fig. 9B). The data suggest that HCl-induced CT responses are elicited by a subpopulation of TRCs contained in different ROIs within the taste bud that contain either an  $\text{H}^+$  entry mechanism in their apical membrane or in their basolateral membrane accessible via paracellular shunts. In contrast for acetic acid, the influx of acid equivalents into TRCs is augmented by a significant flow of unionized acetic acid. This would account for the significantly greater decrease in TRC  $\text{pH}_i$ , consistent with the greater CT response.

## DISCUSSION

Our results show that weak acids enter rat TRCs from the apical side as neutral molecules (acetic acid or dissolved  $\text{CO}_2$ ).  $\text{H}^+$  ions (HCl) also gain access to TRCs, but the pathways involved have not yet been fully elucidated. Irrespective of the mode of acid entry, however, the data indicate that the consequent decrease in TRC  $\text{pH}_i$  serves as the proximate stimulus in sour taste transduction.

*pH<sub>i</sub> as the proximate stimulus in acid detection.* The hypothesis that a decrease in TRC  $\text{pH}_i$  is the proximate stimulus in rat sour taste transduction is borne out by our studies with acetic acid stimuli at pH 6.0 and dissolved  $\text{CO}_2$  as an acid stimulus. Even at pH 6.0, a  $\text{pH}_i$  that is significantly above the threshold pH that gives a detectable CT response, acetic acid was as good a stimulus as at pH 3.0. Acetic acid responses were completely independent of  $\text{pH}_o$  and K-acetate concentration. Our data indicate that the permeability of the undissociated acetic acid is the critical factor in deter-

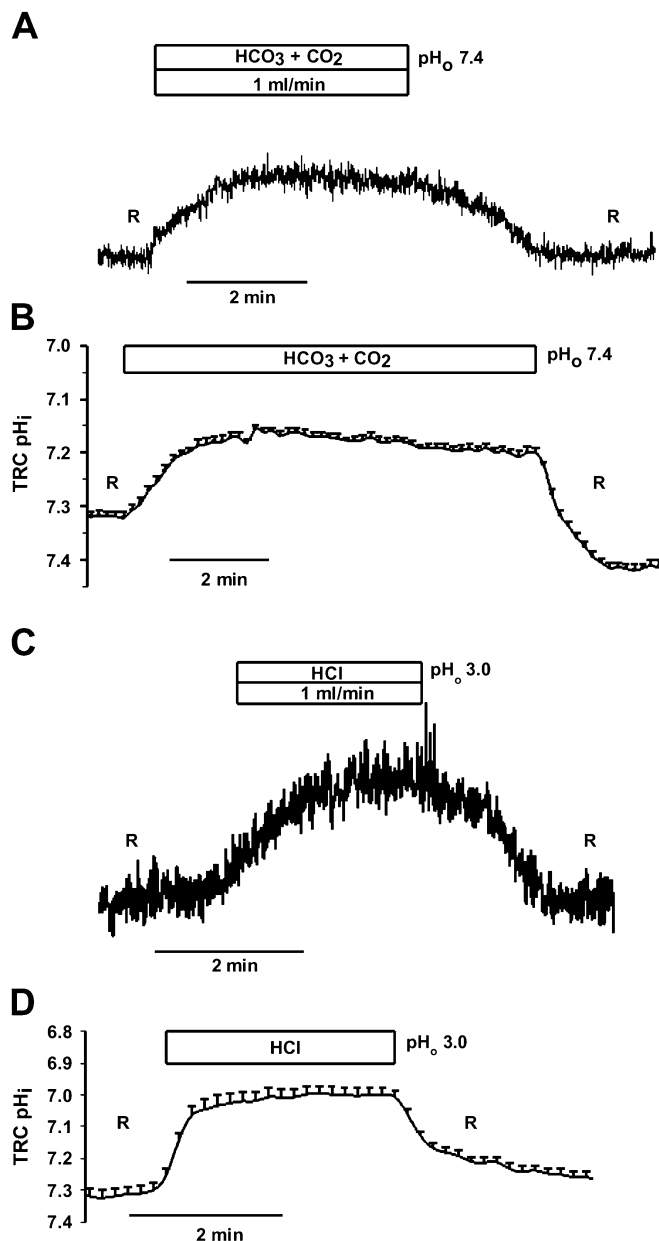


Fig. 8. Temporal relation between CT activity and TRC  $\text{pH}_i$ . The temporal relation between the CT responses (*A*) and the TRC  $\text{pH}_i$  changes (*B*) to dissolved  $\text{CO}_2$  is shown when both the rinse and stimulus were applied at the rate of 1 ml/min. The temporal relation between the CT responses (*C*) and the TRC  $\text{pH}_i$  changes (*D*) to HCl stimulation (pH 3.0) is shown when both the rinse and stimulus were applied at the rate of 1 ml/min.

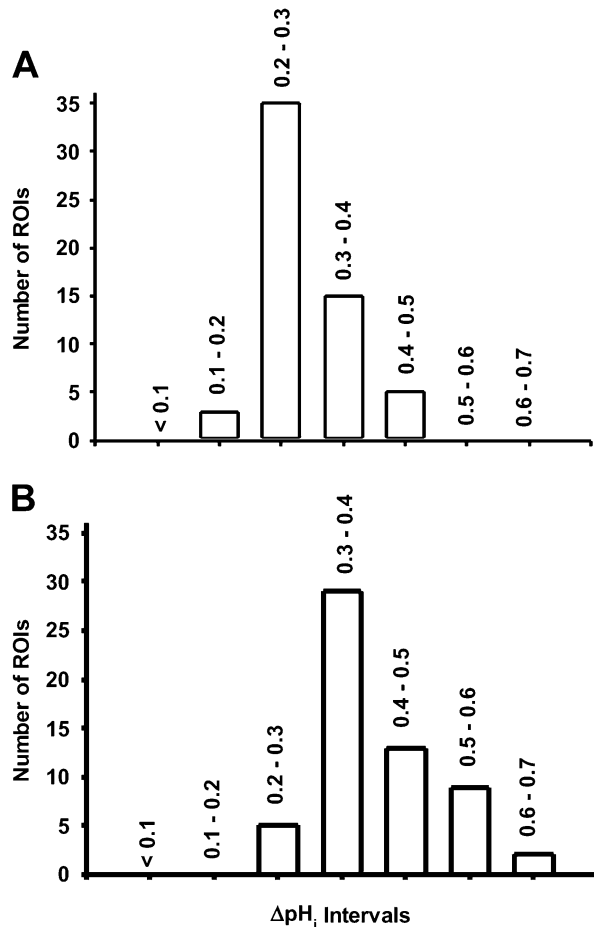


Fig. 9. TRC  $\text{pH}_i$  changes in different ROIs in the taste buds. Three tissues were exposed to apical acetic acid (A) or HCl (B) at pH 3.1, and changes in TRC  $\text{pH}_i$  were monitored in 58 ROIs in the taste buds. For each acid, a histogram shows the number of ROIs that fall within a given  $\text{pH}_i$  interval.

mining its intensity as a stimulus (13, 27). Inside the cell, acetic acid is dissociated into  $\text{H}^+$  and acetate ions. Thus a decrease in TRC  $\text{pH}_i$  is the proximate stimulus for acetic acid-induced increase in CT responses at pH 6.0.

At constant  $\text{pH}_o$ , dissolved  $\text{CO}_2$  decreased TRC  $\text{pH}_i$  and increased CT responses. A membrane-permeable carbonic anhydrase blocker, MK-417, attenuated CT responses and inhibited changes in TRC  $\text{pH}_i$ . It is interesting to note that unlike CT responses to HCl (9, 34) and acetic acid (data not shown), the  $\text{CO}_2$  responses were voltage sensitive. Inside the cell, the dissolved  $\text{CO}_2$  is converted to  $\text{H}_2\text{CO}_3$  in a reaction catalyzed by intracellular carbonic anhydrases. The  $\text{H}_2\text{CO}_3$  yields free  $\text{H}^+$  and  $\text{HCO}_3^-$  ions. It is likely that  $\text{HCO}_3^-$  ions exit TRCs via conductive pathways and/or ion exchangers in the cell membranes (unpublished observations). Because  $\text{CO}_2$  permeability across cell membranes is not voltage sensitive, voltage changes most likely affect CT responses indirectly by modulating the  $\text{HCO}_3^-$  flux across TRC membranes. Because the hydration of  $\text{CO}_2$  leads to the formation of  $\text{H}^+$  and  $\text{HCO}_3^-$  in stoichiometrically equal proportions, alteration in the concentra-

tion of the latter under voltage-clamp conditions will produce immediate changes in  $\text{pH}_i$ . If a decrease in  $\text{pH}_i$  is the necessary precursor to a sour taste response, the time course of the CT response ought to follow that of the  $\text{pH}_i$  when the sour stimulus is applied at a given fixed rate. The optimal means of making this determination would be to measure both  $\text{pH}_i$  and the neural response in the same system simultaneously. For technical reasons, this was not possible. However, when we obtained the  $\text{pH}_i$  profiles and the CT response for both HCl and  $\text{CO}_2$  in separate experiments but at the same stimulus flow rate, their time courses were quite comparable (cf. Fig. 8). This result is consistent with the conclusion that the change in  $\text{pH}_i$  is the actual rate-limiting stimulus in acid taste transduction.

Similar studies in other mammalian pH-sensors have also identified  $\text{pH}_i$  as the proximate stimulus in acid detection. A decrease in  $\text{pH}_i$  induced by elevated levels of  $\text{CO}_2$  depolarized pH-sensing neurons (28, 30, 38) and increased action potential frequency. In cultured ventrolateral medullary neurons [37] during  $\text{NH}_4\text{Cl}$  pulses a decrease in  $\text{pH}_i$  led to increased action potential frequency. A decrease in  $\text{pH}_i$  induced by blocking the neuron membrane  $\text{Na}^+/\text{H}^+$  exchanger resulted in increased cell excitability (38). Acid detection by carotid body type I cells also depends on a decrease in  $\text{pH}_i$  (5, 6). It appears, therefore, that mammalian pH sensory cells have the common property of responding to changes in  $\text{pH}_i$  as the proximate stimulus in acid detection.

*Proton interactions with TRCs.* Our data indicate that  $\text{H}^+$  ions rapidly enter TRCs. Within the taste bud, a subpopulation of TRCs appears to contain an  $\text{H}^+$  entry mechanism either in the apical membranes or in the basolateral membranes made accessible via paracellular shunts. During acid stimulation, this subset of TRCs responds with greater changes in  $\text{pH}_i$  and most likely participates in CT responses elicited by strong, fully dissociated mineral acids. However, the exact nature of the  $\text{H}^+$  entry mechanism remains unknown principally because the effect of  $\text{H}^+$  ions on TRC membrane conductances varies considerably among species (9, 22, 26, 33).  $\text{H}^+$  entry through apical amiloride-sensitive epithelial  $\text{Na}^+$  channels (ENaC) has been suggested as a possible mechanism for acid transduction (14, 22, 33). However, HCl-induced CT responses are not affected by apical  $\text{Na}^+$ , amiloride, or changes in the transepithelial potential of the receptive field under stimulation (9, 34). Similarly, HCl-induced changes in TRC  $\text{pH}_i$  were unaffected by apical  $\text{Na}^+$ , amiloride, or changes in basolateral  $\text{K}^+$  concentration (unpublished observations). In mouse TRCs, acid responses were insensitive to amiloride but were blocked by 5-nitro-2-(3-phenylpropyl-amino)benzoic acid, a  $\text{Cl}^-$  channel blocker (26). Both the mammalian brain  $\text{Na}^+$  channel (BNC1) (36) and the HCN family of channels (32) have been shown to be present in rat vallate TRCs. However, recent evidence suggests that BNC1 is most likely not involved in sour taste transduction (19). Alternatively,  $\text{H}^+$  ions may pass through paracellular

shunts to reach TRC basolateral membrane sites (9). These sites could belong to the HCN channel family.

**pH<sub>i</sub> regulation and pH tracking in TRCs.** The acid-induced changes in TRC pH<sub>i</sub> were sustained. A sustained change in pH<sub>i</sub> is a common feature of acid-sensing cells (30, 37, 38). This, however, does not imply that TRCs lack the ability to regulate pH<sub>i</sub>. We have previously (23, 34) shown that at constant pH<sub>o</sub>, isolated TRCs recover from intracellular acid loading. It is likely that during acid stimulation, the inhibition of pH recovery mechanisms contributes to a sustained decrease in pH<sub>i</sub> (Ref. 30 and unpublished observations). This ensures that for a given acid stimulus, the induced pH<sub>i</sub> changes will be maximal and graded.

In contrast to the apical membrane, the relationship between pH<sub>i</sub> and pH<sub>o</sub> across the basolateral membrane gave a slope of 0.69 (unpublished observations). A similar relationship between pH<sub>i</sub> and pH<sub>o</sub> was observed in isolated TRCs (23, 34). This suggests that the apical membrane of TRCs is significantly less conductive to H<sup>+</sup> ions relative to the basolateral membrane and acts as a filter for H<sup>+</sup> ions. In concert with the paracellular shunt pathway (9, 34), apical cell membranes of TRCs help to regulate H<sup>+</sup> concentration, thereby protecting the sensory apparatus from hyperacidic conditions. Consequently, a large change in apical pH<sub>o</sub> is translated into a relatively small change in pH<sub>i</sub>. This ensures that variations in TRC pH<sub>i</sub> remain within the physiological range during the rigors of acid stimulation (5, 30, 38).

**Role of pH<sub>i</sub> in transduction.** In the case of rat acid-sensitive taste receptors, transduction steps subsequent to the decrease in pH<sub>i</sub> are still unknown. In carotid body type I cells, decrease in pH<sub>i</sub> leads to a conductance increase, membrane depolarization, and an increase in intracellular Ca<sup>2+</sup> concentration (5). In the acid-sensors in the locus coeruleus, the proximate stimulus is still a decrease in intracellular pH, but in this case depolarization results from proton-induced closure of K<sup>+</sup> channels (28). Divergence in the subsequent transduction steps in these cases suggests the possibility of alternate mechanisms leading to depolarization in taste cells. To some extent, one should anticipate this given the diversity of mechanisms that have been proposed for sour taste transduction in various species. For example, in the case of *Necturus*, a decrease in apical pH<sub>o</sub> depolarizes taste cells by closing apical K<sup>+</sup> channels (18). Our preliminary data (unpublished observations) suggest that TRC pH<sub>i</sub> is regulated via intracellular second messengers (e.g., intracellular Ca<sup>2+</sup> concentration and cAMP). This suggests that, similar to other sensory cells, sustained changes in TRC pH<sub>i</sub> during acid transduction must involve activation of intracellular signaling mechanisms that result in depolarization of TRC receptor potential and release of neurotransmitter. This is consistent with the studies of Gilbertson et al. (15) where the contribution of cAMP to the development of acid-responses is also indicated.

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