Acid-induced responses in hamster chorda tympani and intracellular pH tracking by taste receptor cells

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Stewart, Robert E., Vijay Lyall, George M. Feldman, Gerard L. Heck, and John A. DeSimone. Acid-induced responses in hamster chorda tympani and intracellular pH tracking by taste receptor cells. Am. J. Physiol. 275 (Cell Physiol. 44): C227–C238, 1998.—HCl- and NaCl-induced hamster chorda tympani nerve responses were recorded during voltage clamp of the lingual receptive field. Voltage perturbations did not influence responses to HCl. In contrast, responses to NaCl were decreased by submucosal-positive and increased by submucosal-negative voltage clamp. Responses to HCl were insensitive to the Na+ channel blockers, amiloride and benzamil, and to methylisobutylamiloride (MIA), an Na+/H+ exchange blocker. Responses to NaCl were unaffected by MIA but were suppressed by benzamil. Microfluorometric and imaging techniques were used to monitor the relationship between external pH (pHo) and the intracellular pH (pHi) of fungiform papilla taste receptor cells (TRCs) following 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein loading. TRC pHi responded rapidly and monotonically to changes in pHi. This response was unaffected by Na+ removal or the presence of amiloride, benzamil, or MIA. The neural records and the data from isolated TRCs suggest that the principal transduction pathway for acid taste in hamster is similar to that in rat. This may involve the monitoring of changes in TRC pHi, mediated through amiloride-insensitive H+ transport across TRC membranes. This is an example of cell monitoring of environmental pH through pH tracking, i.e., a linear change in pHi, in response to a change in pHo, as has been proposed for carotid bodies. In taste, the H+ transport sites may be concentrated on the basolateral membranes of TRCs and, therefore, are responsive to an attenuated H+ concentration from diffusion of acids across the tight junctions.

lingual voltage clamp; microfluorometry; imaging; fungiform papillae

SOURNESS IS THE TASTE quality that humans typically associate with acidic stimuli (20). The quality and intensity of acidic stimuli vary considerably, depending on H+ concentration, the accompanying anion species, and, in the case of “weak” acids, titratable H+ stored as undissociated acid (8, 25). Several cellular mechanisms for H+ transduction in taste receptor cells (TRCs) of amphibians and mammals have been suggested from results of neurophysiological, electrophysiological, microfluorometric, and imaging studies.

Whole cell and loose patch voltage-clamp studies in situ and in isolated Necturus TRCs have shown that acidic stimuli depolarize and elicit action potentials from taste cells by decreasing a resting, outward K+ conductance, probably by H+ blockage of a voltage-sensitive apical K+ channel (14, 15). Studies on isolated bullfrog TRCs suggest that acids induce a depolarizing current by increasing an H+-gated Ca2+ conductance (22). Electrophysiological recordings from isolated hamster TRCs and from intact hamster taste buds in vitro (10, 11) have provided evidence for H+ transduction by translocation through amiloride-sensitive, apical Na+ channels. In these studies, fast current transients elicited from taste buds by both H+ and Na+ were inhibited by amiloride (10), as were voltage-clamping currents in whole cell patch-clamp studies (11). On the other hand, recordings from the rat chorda tympani nerve during voltage clamp of the lingual receptive field failed to demonstrate either voltage or amiloride sensitivity of HCl taste responses (6). Notably, HCl taste stimuli caused an increase in the lingual transepithelial resistance in rats and the polarity of the transepithelial potential reversed sign on application of rinse solutions or salt stimuli (6). These observations imply that H+ pass through the paracellular pathway and are buffered by fixed anionic sites. Therefore, acid-induced effects on TRCs may not be restricted to the apical cell membrane.

More recently, measurement of intracellular pH (pHi) in isolated rat taste bud fragments (TBFS) by microfluorometry, and in single isolated TRCs by imaging techniques, showed that changes in external pH (pHo) induced parallel changes in TRC pHi (18, 19). The pHi-induced changes in TRC pHi were rapid and monotonic and displayed an average slope close to unity. It was suggested that changes in TRC pHi may be involved in acid taste transduction. Therefore, as in amphibians, considerable diversity in acid transduction mechanisms may exist in mammalian species.

In epithelial cells (17, 23, 24), including hamster TRCs (11), H+ can permeate cell membranes via amiloride-sensitive apical Na+ channels. In the present study, we tested the hypothesis that acid taste transduction involves H+ flux via amiloride-sensitive apical Na+ channels in hamster TRCs by using both in vivo and in vitro approaches. This was accomplished by recording hamster chorda tympani responses to HCl and NaCl under current and voltage clamp and in the presence and absence of amiloride and its analogs. In addition, using microfluorometric and imaging techniques, we investigated the relationship between pHi and TRC pHi in isolated hamster TBFS and single TRCs from fungiform papillae in the presence and absence of amiloride and its analogs. We predicted that, if apical H+ flux contributes appreciably to the stimulation of taste nerves by HCl, taste nerve responses to HCl should be modulated by voltage clamp of the lingual receptive field and suppressed by amiloride and its analogs in a manner analogous to that observed with Na+ salts. In addition, amiloride and its analogs should significantly
affect $p_{Hi}$ and the $p_{Hi}$ vs. $p_{He}$ relation observed in isolated TRCs (18).

We found that HCl caused a concentration-dependent increase in hamster chorda tympani responses. However, chorda tympani responses to HCl, at concentrations comparable to those used to obtain NaCl responses, were virtually insensitive both to voltage clamping and to amiloride or its analogs. In the subset of isolated 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF)-loaded hamster TRCs investigated, $p_{Hi}$ showed a strong, linear dependence on $p_{He}$ that was unaffected by amiloride and its analogs. This $pH$ tracking behavior is similar to that observed in rat TRCs (18) and analogous to that reported for $pH$-sensing carotid body type 1 cells (2). This further suggests that changes in TRC $p_{Hi}$ may be involved generally in $H^+$ detection. Moreover, the data suggest that hamster fungiform papilla taste cells, like those of rat (6), transduce $H^+$ stimuli at a site below the taste bud tight junction.

**MATERIALS AND METHODS**

**Recording Chorda Tympani Responses to Acid**

Stimuli. The rinse solution contained 15 mM KHCO$_3$ and 20 mM KCl (pH 8.3). A Na$^+$-depleted Krebs-Henseleit solution (DKH) containing (in mM) 6 KCl, 2 CaCl$_2$, 1.2 MgSO$_4$, 1.3 NaH$_2$PO$_4$, 25 NaHCO$_3$, and 5.6 glucose (pH 7.5) was applied after each stimulus series to maintain a stable transepithelial potential. The transepithelial potential in the presence of 150 mM NaCl rarely varied more than $\pm 5$ mV during an experiment. Stimulus series were with 1 and 10 mM HCl and 10 and 100 mM NaCl.

Nerve preparation and recording. Neural responses were recorded from the chorda tympani nerves of male Syrian Golden hamsters (Charles River Laboratories; 70–120 days of age) during chemical stimulation of the tongue. Hamsters were anesthetized by brief exposure to ether followed by intraperitoneal injection of pentobarbital (90 mg/kg). Supplementary pentobarbital (30 mg/kg) was administered as necessary to maintain surgical anesthesia. Body temperature was maintained at 36–37°C with a circulating water heating pad. The left chorda tympani was exposed laterally as it exited the tympanic bulla, as previously described (13). After the chorda tympani was dissected free from surrounding tissue, it was cut proximally, desheathed, and placed onto a 32G platinum-iridium wire electrode. An indifferent electrode was placed in nearby tissue.

Simultaneous epithelial voltage clamping and chorda tympani neural recordings have been described (31). Stimulus solutions and rinses were injected (4 ml; 1 ml/s) into a Lucite chamber affixed by vacuum to a 28-mm$^2$ patch of the anterior dorsal lingual surface. The chamber was fitted with separate Ag-AgCl electrodes for measurement of current and potential, and reference electrodes were placed noninvasively on the ventral lingual epithelium. The current-passing electrode within the chamber served as a virtual ground, ensuring that only current passing through the stimulated patch was collected. Neural responses were differentially amplified with a custom-built, optically coupled isolation amplifier, then full-wave rectified and integrated with a time constant of 1 s. Integrated neural responses and voltage and current records were recorded on a Linseis TYP7045 chart recorder (Princeton Junction, NJ).

Stimuli were applied under zero current clamp, and steady-state potentials were recorded from the display of the voltage-current clamp amplifier (Physiologic Instruments VCC600, San Diego, CA). Chorda tympani responses were then obtained under voltage clamp at $+60$ and $-60$ mV relative to the zero current clamp potential recorded for each stimulus. Each stimulus series was bracketed by application of 150 mM NaCl under zero current clamp. Neural data were excluded when bracketing responses varied by more than 10%. Steady-state response magnitudes, the height of each integrated chorda tympani response 30 s after application of the stimulus, were expressed relative to the mean 150 mM NaCl response magnitude bracketing a stimulus.

The protocol for stimulation of the lingual epithelium was as follows. First, the reference stimulus (denoted $R_N$) was applied and allowed to remain on the tongue for 40–50 s. The reference solution was then rinsed with several applications (typically three) of the KHCO$_3$-KCl solution (denoted $R_0$) for a total of at least 60 s. Next, the stimulus of interest was applied as a concentration series (denoted $S_1$ and $S_2$), with each allowed to remain in the chamber for 40–50 s. Individual stimulus applications were followed by rinsing as described above. After the second stimulus in a series was rinsed, the reference stimulus was reapplied and rinsed. At this point, DKH was applied, allowed to remain on the tongue for 60 s, and then rinsed. Finally, the reference stimulus was applied and then rinsed, and the next concentration series was started. This protocol can be represented as follows

$$R_N—R_0—S_1—R_0—R_N—R_0—S_2—R_0—R_N—R_0—DKH$$

The voltage-sensitivity index (VSI) was used as a measure of the relative voltage dependence of chorda tympani responses to various stimuli (6, 32). The VSI was defined as

$$VSI = R(C, -60) - R(C, +60)$$

where $R(C, -60)$ is the mean chorda tympani response magnitude at concentration $C$ and clamp voltage of $-60$ mV and $R(C, +60)$ is the mean response at the same concentration and clamp voltage of $+60$ mV.

Chorda tympani responses were also obtained with conventional recording techniques (i.e., without voltage clamping) in a separate set of hamsters. Chorda tympani responses to 100 mM NaCl were obtained in the absence and presence of 5 µM benzamil, a specific Na$^+$ channel blocker, or 1 µM methylisobutylamiloride (MIA), a specific Na$^+/H^+$ exchange blocker (both from Research Biochemicals International, Natick, MA) (17). In addition, responses to 1 and 10 mM HCl were obtained in the absence and presence of 50 µM amiloride (Sigma Chemical, St. Louis, MO) or 5 µM benzamil or 1 µM MIA. Responses to these NaCl and HCl solutions were expressed relative to responses to 300 mM NH$_4$Cl. Solutions containing amiloride, benzamil, or MIA were made freshly each day from frozen stock solutions as described in Composition of Solutions.

**pHi Measurements in Isolated TBFs**

Preparation of TBFs. Hamsters were anesthetized with ether and then killed by cervical dislocation. The tongue was rapidly removed and stored in ice-cold HEPES-buffered Tyrode solution (pH 7.4) preequilibrated with 100% O$_2$. Fungiform TBFs were prepared by the conventional collagenase (Boehringer Mannheim, Indianapolis, IN) method. The details of the method and the tests used to determine the viability of TRCs in isolated TBFs were as described previously (18).
Perfusion chamber. The open perfusion chamber consisted of a standard glass slide on which a piece of Silastic sheet (with a 4-cm² cutout window in the center) was glued (18). An infusion pump delivered solutions (4 ml/min) into the chamber through ports located in three sides of the chamber. The chamber surface was precoated with Cell-Tak (1 µg/cm²; Collaborative Research, Bedford, MA) to affix the cells. Fluid exchange, measured as BCECF (Molecular Probes, Eugene, OR)-acid washout from the chamber, had an initial rapid phase, during which 70% of the dye was cleared from the chamber in 4 s (n = 5), and a slower phase, during which another 20% of dye was cleared in 33 s (18). All tubing was thoroughly washed with distilled water after each experiment. In addition, a fresh chamber was made for each experiment by attaching the Silastic sheet to a new glass slide precoated with Cell-Tak.

pH i measurements. TBFs were loaded with BCECF by incubation in HEPES-buffered Tyrode solution (pH 7.4) containing 30 µM BCECF-AM (Molecular Probes) for 1 h in the dark at 4°C. TBFs were then transferred to the Cell-Tak-coated perfusion chamber and washed 15 min later by perfusion with 30 ml of HEPES-buffered Tyrode solution. As described earlier (18), both microfluorometric and imaging techniques were used to measure pH i in isolated TBFs. Briefly, the BCECF-loaded TBFs and single isolated TRCs were observed through a Zeiss ×40 objective (numerical aperture = 0.9). Excitation light was alternated between 440 and 490 nm using a filter wheel in the microfluorometry system and a slider in the imaging system. The dichroic beam splitter and emission filters were 515 and 535 nm, respectively. Filters were obtained from Omega Optical. In the imaging system, each image was constructed from the average of eight frames. The pH i was determined from the ratio of fluorescence emission intensities, F 490/F440 ratio, where F is fluorescence, and the signals were calibrated using high-K + plus nigericin solutions. All measurements were made at room temperature (22 ± 1°C).

As in our previous study (18), measurements of pH i on isolated TBFs were accepted only if the following criteria were met: 1) the TRCs retained the BCECF throughout the experiment and 2) the TBFs gave a satisfactory pH i calibration with nigericin-containing, high-K + calibrating solutions. All measurements were made at room temperature (22 ± 1°C).

Data are expressed as mean ± SE, unless indicated otherwise. Potential-dependent differences in chorda tympani response magnitudes were assessed using ANOVA with Student-Newman-Keuls posttests (SNK) where appropriate. Differences between response magnitudes with and without Na + channel and antiporter blockers were determined using paired Student's t-tests. Differences in TRC pH i were analyzed using both paired and unpaired Student's t-tests. The slopes and correlation coefficients of the least squares regression lines drawn through data plots of pH i vs. pH e are represented by S and r, respectively.

RESULTS

Chorda Tympani Responses to HCl and NaCl Under Voltage Clamp

Figure 1 shows chorda tympani responses to 10 mM NaCl and to 1 and 10 mM HCl under zero current clamp and ±60-mV voltage clamp. Chorda tympani responses to 10 mM NaCl were suppressed under...
submucosal-positive voltage clamp, whereas those obtained under submucosal-negative voltage clamp were elevated relative to responses at zero current clamp. Similar, but more pronounced, changes in chorda tympani response magnitude under voltage clamp were observed with 100 mM NaCl. ANOVA with post hoc SNK posttests revealed, for both 10 mM (Fig. 2) and 100 mM NaCl, that chorda tympani responses under positive voltage clamp were significantly suppressed and those under negative voltage clamp were significantly elevated vs. responses obtained under zero current clamp [with 10 mM: \( F(2,12) = 21.60, P < 0.0001 \); with 100 mM: \( F(2,11) = 88.90, P < 0.001 \); posttest \( P \) values < 0.05]. Figure 1 also shows chorda tympani responses to 1 and 10 mM HCl obtained from the same hamster. Lingual receptive field voltage perturbations had little impact on the magnitude of chorda tympani responses to HCl. Indeed, no significant effect of voltage-clamp condition on chorda tympani response magnitudes to either 1 or 10 mM HCl was detected [ANOVA \( F(2,10) = 1.87, P \) values ≥ 0.21] (Fig. 2). This lack of HCl response voltage sensitivity is illustrated further by calculations of VSI s for each stimulus (Table 1).

As expected, positive VSIs for NaCl stimuli were obtained, reflecting the voltage dependence of Na⁺ movement into taste cells via apical Na⁺ channels. In contrast, VSIs for HCl stimuli were both close to zero. The ratio of the VSI for 10 mM NaCl to that for 10 mM HCl is 16.6, whereas the ratio of the VSIs for 100 mM NaCl to 10 mM HCl is 36.8. That is, chorda tympani responses to 10 and 100 mM NaCl are, respectively, 17 and 37 times more sensitive to voltage perturbation than are those to 10 mM HCl.

Another feature of the hamster HCl taste response is the transmural potential response to HCl application and rinse. Figure 3 depicts integrated chorda tympani responses and corresponding potentials evoked under zero current clamp by 10 mM HCl and 100 mM NaCl. The potential attending application of 100 mM NaCl was reminiscent of those observed in the rat on application of Na⁺ stimuli to the lingual receptive field.

![Fig. 2. Mean chorda tympani relative response magnitudes for 1 and 10 mM HCl and 10 mM NaCl obtained with zero current clamp and with +60- or -60-mV voltage clamp. Responses to either concentration of HCl showed no significant sensitivity to applied voltage perturbations. In contrast, responses to 10 mM NaCl were significantly suppressed under positive voltage clamp (relative to both zero current clamp and positive voltage clamp) \( n = 6 \).](image)

**Table 1. Voltage sensitivity indexes for NaCl and HCl**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Voltage Sensitivity Index</th>
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<tr>
<td>NaCl</td>
<td></td>
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<tr>
<td>10 mM</td>
<td>0.414 ± 0.060</td>
</tr>
<tr>
<td>100 mM</td>
<td>0.923 ± 0.074</td>
</tr>
<tr>
<td>HCl</td>
<td></td>
</tr>
<tr>
<td>1 mM</td>
<td>-0.033 ± 0.003</td>
</tr>
<tr>
<td>10 mM</td>
<td>0.025 ± 0.026</td>
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Values are means ± SE; \( n = 6 \).

![Fig. 3. Integrated chorda tympani (B) and corresponding evoked transepithelial potential (A) responses to 10 mM HCl and 100 mM NaCl. Potentials evoked by 10 mM HCl exhibited an initial rising phase, followed by a broad rising phase. When rinse (R) was applied, a brief potential reversal was observed, followed by a slow return to baseline values. This potential reversal and slow relaxation to baseline corresponded well with large rinse transients and persistent elevated activity in the neural response on and following rinse of HCl. In comparison, 100 mM NaCl application was attended by a rapid, positive change in potential to a pseudo-steady-state value that paralleled the tonic phase of the chorda tympani response. After rinse with NaCl, transepithelial potential returned abruptly to resting values. Voltage scale applies only to potential records.](image)
Specifically, there was a rapid, inwardly directed positive change in potential followed by a pseudo-steady-state potential that paralleled the tonic phase of the chorda tympani response. After rinse with NaCl, the potential abruptly returned to resting value after a brief overshoot. In comparison, the positive potential change in response to application of 10 mM HCl had a somewhat slower initial ascending phase followed by a broad, slowly rising component. After rinse with HCl, a sizable, positive-going potential was observed, followed by a slow return to baseline potential values. It is notable that the duration of the postrinse "afterpotential" seemed to be highly correlated temporally with persistent elevated baseline neural activity that was frequently observed after rinsing HCl stimuli. These data are consistent with the HCl-induced responses in rats reported earlier by DeSimone et al. (6).

Sensitivity of Chorda Tympani HCl and NaCl Responses to Amiloride and Its Analogs

As expected, chorda tympani responses to 100 mM NaCl were significantly suppressed when this stimulus was dissolved in 5 µM benzamil (Student's t = 5.92, P < 0.002) (Figs. 4 and 5). In comparison, 1 µM MIA had no measurable effect on chorda tympani responses to 100 mM NaCl (Student's t = 1.10, P = 0.35). Also, 50 µM amiloride, 5 µM benzamil, and 1 µM MIA did not affect the magnitude of chorda tympani responses to 1 or 10 mM HCl (P values > 0.12). However, in three preparations, considerable (up to 50%) suppression of 10 mM HCl responses by benzamil was observed, whereas in no cases were responses to HCl suppressed by amiloride. Suppression by benzamil occurred in preparations that had unusually large chorda tympani responses to 10 mM HCl (up to twice the mean relative response magnitude to this stimulus).

Characteristics and Pharmacology of pH Tracking by TRCs

In typical preparations, hamster fungiform papilla TBFs (Fig. 6A) and individual TRCs were readily identified (Fig. 7A). Occasionally, some TRCs were rounded. In some taste buds, BCECF was distributed in most, if not all, cells (18). These cases were normally selected for further study. In individual TRCs, BCECF fluorescence was intracellular (Fig. 7B). The TBFs and TRCs retained the dye without significant loss for several hours, were then calibrated satisfactorily, and were determined to be viable and functional by criteria described elsewhere (18). As done previously, microfluorometric measurement of pH_i was carried out exclusively on a subset of TBFs in which the dye appeared to be distributed in most cells (18). In single, isolated TRCs, pH_i was measured by imaging (see Fig. 7).

Effect of changes in pH_o on pH_i. At pH_o of 7.4, the mean resting TRC pH_i was 7.46 ± 0.04 (n = 6). This
value compares well with the resting values of rat TRC pH measured under identical conditions (18). Figure 8 shows the effect of step changes in pHo on TBF pHi. When the TBF was initially perfused with HEPES-buffered Tyrode solution at pH 7.42, the cells maintained a mean resting pHi near 7.4. If pHo was decreased to 6.78 and subsequently raised to 7.87, pHi responded rapidly and stabilized at values near 6.8 and 8.0, respectively. When pHo was returned to 7.42, pHi promptly returned to a value close to 7.4. Figure 9 shows the relation of pHi to pHo in six TBFs. The S of the regression lines of pHo vs. pHi plotted for individual TBFs varied between 0.94 and 1.24 and gave a mean value of 1.08 ± 0.04. The mean r was 0.996 ± 0.003. The maximum rates of change in pHi (maximum ΔpHi/ min) for different pHo steps varied between 1.8 and 4.3 and depended on the pHo gradient (Table 2). The pHo-induced changes in pHi were monotonic and stable. Overall, pHo-induced changes in hamster fungiform TBF pHi were very similar to those seen in rat TBFs (18).

Effect of amiloride on pH tracking. Figure 10 shows the effect of 100 µM amiloride on the pHo-induced change in TBF pHi. Exposure to amiloride induced a small, significant decrease in resting TBF pHi. In four paired experiments in the presence of amiloride, pHi was 7.42 ± 0.06, a value significantly lower than the pHi of 7.50 ± 0.06 measured in its absence (ΔpHi = 0.08 ± 0.03, P < 0.05, paired). However, as shown in Fig. 10, the pHo-induced changes in TBF pHi were not affected by amiloride. The relationship between pHo and pHi in four paired experiments is also shown in Fig. 9. The S of the regression lines of pHo vs. pHi plots for individual TBFs varied between 0.99 and 1.24, with a mean of 1.10 ± 0.05 (mean r = 0.996 ± 0.003). As also shown in Table 2, amiloride did not affect the maximum rates of change in pHi (maximum ΔpHi/min) for different pHo steps.

Effect of Na+ substitution. Figure 11 shows the effect of Na+ removal on pHo-induced effects on TBF pHi. Replacement of Na+ with NMDG+ induced a small decrease in resting TBF pHi. In four paired experiments, the pHi in the absence of Na+ was 7.38 ± 0.04, a value significantly lower than the pHi of 7.50 ± 0.06 in control medium (ΔpHi = 0.11 ± 0.04, P < 0.05, paired). However, pHo-induced changes in TBF pHi were not
affected by Na\(^+\) removal (Fig. 11). The relationship between pH\(_o\) and pH\(_i\) in four paired experiments in the absence of Na\(^+\) is plotted in Fig. 9. The S of the regression lines of pH\(_o\) vs. pH\(_i\) plotted for individual TBFs varied between 0.92 and 1.14 and gave a mean of 1.05 ± 0.05 (mean r = 0.996 ± 0.001). As also shown in Table 2, Na\(^+\) removal did not affect the maximum rates

![Pseudocolor ratio images of TRCs loaded with BCECF. A: an optical section through at least 2 fungiform TRCs. Scale bar, 5 μm. B: same optical section is shown as BCECF fluorescence image, excited at the pH-sensitive wavelength, 490 nm. Note that the dye distribution pattern reflects the irregular shapes of the TRCs. Same optical section is shown as the ratio of BCECF fluorescence pseudocolor images when excited alternately at 490 and 440 nm at the steady-state pH\(_o\) of 6.6 (C) and 7.7 (D). Note that a decrease in pH\(_o\) induced parallel decreases in pH\(_i\) in both the soma and apical processes.](image1)

![Fig. 7. Pseudocolor ratio images of TRCs loaded with BCECF. A: an optical section through at least 2 fungiform TRCs. Scale bar, 5 μm. B: same optical section is shown as BCECF fluorescence image, excited at the pH-sensitive wavelength, 490 nm. Note that the dye distribution pattern reflects the irregular shapes of the TRCs. Same optical section is shown as the ratio of BCECF fluorescence pseudocolor images when excited alternately at 490 and 440 nm at the steady-state pH\(_o\) of 6.6 (C) and 7.7 (D). Note that a decrease in pH\(_o\) induced parallel decreases in pH\(_i\) in both the soma and apical processes.](image2)

Fig. 7. Pseudocolor ratio images of TRCs loaded with BCECF. A: an optical section through at least 2 fungiform TRCs. Scale bar, 5 μm. B: same optical section is shown as BCECF fluorescence image, excited at the pH-sensitive wavelength, 490 nm. Note that the dye distribution pattern reflects the irregular shapes of the TRCs. Same optical section is shown as the ratio of BCECF fluorescence pseudocolor images when excited alternately at 490 and 440 nm at the steady-state pH\(_o\) of 6.6 (C) and 7.7 (D). Note that a decrease in pH\(_o\) induced parallel decreases in pH\(_i\) in both the soma and apical processes.

![Fig. 8. Effect of changing pH\(_o\) on pH\(_i\). A TBF was perfused with Tyrode solution of pH 7.42, 6.78, and 7.87. Top horizontal bar represents periods during which the TBF was perfused with solutions of different pHs.](image3)

![Fig. 8. Effect of changing pH\(_o\) on pH\(_i\). A TBF was perfused with Tyrode solution of pH 7.42, 6.78, and 7.87. Top horizontal bar represents periods during which the TBF was perfused with solutions of different pHs.](image4)

![Fig. 9. Relationship between pH\(_o\) and pH\(_i\). Steady-state relationship between pH\(_o\) and pH\(_i\) in 5 TBFs under control conditions (●). Line of best fit determined by linear regression (S = 1.08 ± 0.04; r = 0.996 ± 0.003). Steady-state relationship between pH\(_o\) and pH\(_i\) in 4 paired TBFs in the presence of 0.1 mM amiloride (▲: S = 1.10 ± 0.05; r = 0.996 ± 0.003). Steady-state relationship between pH\(_o\) and pH\(_i\) in 4 paired TBFs in the absence of external Na\(^+\) (■: S = 1.05 ± 0.05; r = 0.996 ± 0.001).](image5)

![Fig. 9. Relationship between pH\(_o\) and pH\(_i\). Steady-state relationship between pH\(_o\) and pH\(_i\) in 5 TBFs under control conditions (●). Line of best fit determined by linear regression (S = 1.08 ± 0.04; r = 0.996 ± 0.003). Steady-state relationship between pH\(_o\) and pH\(_i\) in 4 paired TBFs in the presence of 0.1 mM amiloride (▲: S = 1.10 ± 0.05; r = 0.996 ± 0.003). Steady-state relationship between pH\(_o\) and pH\(_i\) in 4 paired TBFs in the absence of external Na\(^+\) (■: S = 1.05 ± 0.05; r = 0.996 ± 0.001).](image6)
of change in pH (maximum ΔpH/min) for different pH₀ steps.

In three additional experiments, TBFs were bathed in Na⁺-free solutions containing 100 µM amiloride. In the continuous presence of amiloride, the pH₀-induced changes in TRC pHᵢ were not different from those observed in TRCs bathed in Na⁺-free solutions without amiloride, as shown in Figs. 9 and 10.

Effect of weak acids. To determine if the neutral forms of weak acids are membrane permeable and contribute to changes in pHᵢ, TBFs were perfused with Tyrode solutions containing 30 mM sodium acetate at constant pH₀. TBFs responded to sodium acetate solution with a rapid intracellular acidification followed by a spontaneous recovery of pHᵢ (Fig. 12). When sodium acetate was subsequently removed, there was a rapid intracellular alkalinization followed by a recovery phase. Similar changes in pHᵢ were observed in two additional TBFs exposed to sodium acetate. These data are consistent with the effects of weak acids on rat TBFs (18) and suggest that TRCs behave like many other cells: 1) the TRC membranes are intact and are able to partition undissociated and dissociated species of weak acids; 2)

Changes in TRC pH, Monitored by Imaging

To determine whether pH₀ has regional effects on pHᵢ of TBFs and isolated TRCs, imaging studies were performed. Images of an isolated fungiform papilla TBF were acquired within the same optical plane at pH₀ of 7.77, 7.39, and 6.78. The acquired images were arbitrarily divided into 20 small regions of interest (ROIs; 3 × 3 µm). Each ROI was individually calibrated and served as its own control (18). For example, at pH₀

Table 2. Maximum rates of change in pHᵢ induced by step changes in pH₀

<table>
<thead>
<tr>
<th>Step Change in pH₀</th>
<th>Maximum ΔpHᵢ/min</th>
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<tbody>
<tr>
<td>7.4–6.7</td>
<td>-2.0 ± 0.2</td>
</tr>
<tr>
<td>6.7–7.8</td>
<td>4.3 ± 0.6</td>
</tr>
<tr>
<td>7.8–7.4</td>
<td>-1.8 ± 0.2</td>
</tr>
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</table>

Values are means ± SE of indicated n. Taste bud fragments were perfused with HEPES-buffered Tyrode solutions of different pHs. Maximum ΔpHᵢ/min, maximum rate of change in intracellular pH (pHᵢ); pH₀, external pH.

Fig. 10. Effect of changing pH₀ on pHᵢ in the presence of 0.1 mM amiloride. A TBF was initially perfused with Tyrode solution of pH 7.48. This was followed by perfusion with solutions of pH 6.82, and 7.89 in the continuous presence of 100 µM amiloride. Top horizontal bar represents periods during which the TBF was perfused with solutions of different pHs.

Fig. 11. Effect of changing pH₀ on pHᵢ in the absence of external Na⁺. A TBF was initially perfused with Tyrode solution of pH 7.40. This was followed by perfusion with Na⁺-free solutions of pH 7.38, 6.68, and 7.79. Top horizontal bar represents periods during which the TBF was perfused with solutions of different pHs.

BCECF is intracellular and reflects changes in pHᵢ and 3) at iso-pH₀ TRCs exposed to weak acids can regulate pHᵢ.

Changes in TRC pH, Monitored by Imaging

To determine whether pH₀ has regional effects on pHᵢ of TBFs and isolated TRCs, imaging studies were performed. Images of an isolated fungiform papilla TBF were acquired within the same optical plane at pH₀ of 7.77, 7.39, and 6.78. The acquired images were arbitrarily divided into 20 small regions of interest (ROIs; 3 × 3 µm). Each ROI was individually calibrated and served as its own control (18). For example, at pH₀

Fig. 12. Effect of sodium acetate on TRC pHᵢ. A TBF initially perfused with control Tyrode solution was exposed to a similar Tyrode solution containing 30 mM sodium acetate. Top horizontal bar represents periods during which the TBF was perfused with solutions with and without acetate. Broken lines represent the time periods during which the TBFs were not exposed to excitation light to prevent quenching of the dye.
of 7.77, the TBF pH in individual ROIs varied between 7.47 and 8.3 (mean = 7.95 ± 0.06). At pHo of 7.39, the pH varied between 7.04 and 7.66 (mean = 7.40 ± 0.033), and, at pHo of 6.78, the pH varied between 6.79 and 6.96 (mean = 6.88 ± 0.01). The relationship between pH and mean pH in individual ROIs was linear. Within individual ROIs, the r values of the regression between pHo and pH varied between 0.942 and 0.999 (mean = 0.973 ± 0.004), whereas S of the regression lines varied between 0.51 and 1.42 (mean = 1.06 ± 0.05). In five TBFs, the relationship between pHo and mean pH was also linear and had mean S values that varied between 0.73 to 1.22 (mean = 0.96 ± 0.09; r = 0.992 ± 0.005). This relationship between pHo and pH is similar to that observed microfluorometrically (Fig. 9). Finally, Fig. 7, A-D, shows the pH-induced changes in pHr in two fungiform papilla TRCs. The TRCs responded to a decrease in pH with a parallel decrease in pHr in both the soma and apical processes.

In fungiform papilla TBFs, the relationship between pHr vs. pH in 20 individual ROIs also remained linear in the presence of 5 µM benzamil. The S of the relationship between pHo and pH in individual ROIs varied between 0.89 and 1.29 (mean S = 1.03 ± 0.04), and the r values varied between 0.95 and 0.99 (mean r = 0.985 ± 0.003). These values were not significantly different from those obtained under control conditions. In additional, in paired experiments in three isolated TRCs exposed to 1 µM MIA or 5 µM benzamil, the mean S of the relationship between pHo and pHo was 1.04 ± 0.08 (mean r = 0.98 ± 0.008). These results are consistent with data obtained microfluorometrically, which demonstrate no significant effect of amiloride on the relationship between pHo and pHr in TBFs (Fig. 9).

**DISCUSSION**

**Chorda Tympani Response to NaCl**

The amiloride-sensitive Na+ channel has been implicated in the transduction of Na+ salt taste stimuli in every mammalian species examined (28). Using in vivo receptive field voltage-clamp methods, Ye et al. (30, 31) demonstrated that the amplitude of rat chorda tympani responses to Na+ salt stimuli can be modulated by alteration of the driving force for Na+ across the taste cell apical membrane. The present results extend those findings by showing that hamster chorda tympani responses to NaCl are also highly sensitive to voltage perturbations applied to the lingual receptive field (29). We conclude that the appropriate stimulus dimension for Na+ taste stimuli in hamster is the electrochemical concentration, as previously demonstrated in rat (29, 30).

**Chorda Tympani Response to HCl**

Protons have been shown to permeate amiloride-sensitive Na+ channels in epithelial cells (17, 23, 24), including hamster fungiform papilla TRCs (10, 11). In the present work, we conducted experiments to probe further the hypothesis that apical, amiloride-sensitive Na+ channels are a major transduction site for acid stimuli in hamster fungiform papilla taste cells. Four specific experimental predictions were tested. First, hamster chorda tympani responses to HCl should be modulated by applied voltage perturbations, i.e., responses to HCl should be elevated under negative voltage clamp and suppressed under positive voltage clamp in a manner similar to NaCl. Second, chorda tympani responses should be inhibited by apical Na+ channel blockers, amiloride and benzamil, in a manner similar to NaCl response inhibition. Third, resting steady-state TRC pHr should be sensitive to amiloride and benzamil. Fourth, if pHr-induced changes in TRC pH have a steep relationship similar to that observed in rat TRCs (18, 19), then the S of this relationship should decrease in the presence of amiloride or benzamil.

**Voltage Sensitivity in HCl Chorda Tympani Responses**

Chorda tympani recordings show that, in general, HCl responses were not significantly influenced by voltage perturbation. This was unexpected given the significant pH-induced currents and conductance increases observed in whole taste buds (10) and single taste cells (11) in in vitro electrophysiological recordings. Using 10 mM NaCl, we observed a highly voltage-sensitive chorda tympani response; however, the chorda tympani response to 10 mM HCl was voltage insensitive. Given the conductance measurements reported from whole cell recordings on hamster taste cells (11) and taste buds in situ (10) and with the use of the Goldman-Hodgkin-Katz ion flux equation, the expected channel conductance for the case of a mucosal-side stimulus consisting of 10 mM H+ relative to that for a 10 mM Na+ stimulus can be estimated. The conductance (g) ratio (gH to gNa) is estimated to be at least 25 for intracellular potentials less than zero, consistent with the reported permeability ratio for the channel (10). The expected proton influx through the apical amiloride-sensitive channels should be, therefore, greater than that for Na+ at the same concentration. Were this proton traffic to cause taste nerve excitation, the voltage sensitivity in the chorda tympani response to HCl would be comparable to that observed with NaCl and, therefore, easily detectable in our whole nerve recordings, especially in recordings made under variable lingual voltage-clamp conditions. That this is not so requires some further consideration.

It is possible that two populations of chorda tympani fibers contribute to the whole nerve response to acids. One population of axons innervates taste cells that possess apical, amiloride-sensitive Na+ channels (i.e., N fibers), whereas the other population innervates taste cells that lack apical Na+ channels (i.e., H fibers) (13). It is conceivable, therefore, that in the presence of a dominant H fiber response component, the N fiber (i.e., voltage and amiloride sensitive) component of acid responses could be obscured. However, this is unlikely because, in the hamster chorda tympani, N fibers comprise a much larger segment of the total fiber population than do H fibers (13). It follows that a larger...
proportion of the whole nerve response to acids should be contributed by N fibers than by H fibers. Because the conductance of protons through the apical, amiloride-sensitive Na\(^+\) channels is expected to be greater than that of an equal concentration of Na\(^+\), there is little reason to believe a priori that protons would activate a smaller population of N fibers than would Na\(^+\). On the other hand, it is possible that voltage sensitivity to protons is present only in the early transient of the N fiber response, i.e., voltage sensitivity is a time-dependent parameter. However, recordings from whole hamster taste buds (10) and single taste cells (11) in vitro clearly show pH-induced, amiloride-sensitive currents that were sustained for tens of seconds. In addition, the present data indicate that neither voltage perturbations nor pharmacological probes altered the transient part of the chorda tympani response to HCl (Figs. 1 and 4). The virtual absence of an observable N fiber contribution to the HCl response, despite the high proton conductance of the apical, amiloride-sensitive Na\(^+\) channels, suggests that some intervening process limits excitation of N fiber taste afferents when their associated taste cells are stimulated by acids. This is consistent with hamster chorda tympani single-unit studies that show acids in general to be poor N fiber stimuli. Although in a minority of N fibers HCl is a better stimulus than other acids, in two-thirds of the N fibers HCl is as poor a stimulus as other acids (13). It will, nonetheless, be important and revealing to determine if voltage- and amiloride-sensitive acid responses are detectable in some single chorda tympani axons and, if so, how such responses differ quantitatively from those evoked by Na\(^+\) salt stimuli.

**Effect of Amiloride Analogs**

In general, HCl chorda tympani responses in hamster were not significantly inhibited by amiloride or benzamil, similar to results for rat (6). Consistent with these results, responses of neurons in the hamster nucleus of the solitary tract (NST) to anterior lingual stimulation with 3 mM HCl were not affected by lingual preadaption with 10 µM amiloride (26). It must be noted, however, that suppression by amiloride of hamster chorda tympani HCl responses has been reported. However, it was either not statistically significant (12) or was observed only inconsistently (13). We also noted an aspect of this inconsistency because in three preparations benzamil (but not amiloride) suppressed HCl responses.

Hettinger and Frank (13) suggested that some of the inconsistencies in the suppression by amiloride of single chorda tympani neuron responses to HCl could be attributed to "old" preparations that had been stimulated repeatedly and, therefore, to progressive degradation of Na\(^+\) channels. However, in our three exceptional cases, the preparations were subjected to neither unusually numerous HCl stimulations nor unusually lengthy recording durations. Instead, a common feature in these three cases was an unusually large baseline HCl response. Another aspect regarding amiloride effects on acid responses is the mode of application of amiloride during an experiment.

In a recent study (1), acid responses recorded from hamster NST neurons that were classified as "NaCl-best" were suppressed when acidic stimuli were dissolved in 10 µM amiloride and applied during the tonic phase of the response to the stimulus without amiloride. This finding contrasts with earlier results that showed that preadaptation with amiloride did not significantly inhibit the response to acid of such neurons (26). These conflicting results suggest that detection of a suppressive effect of amiloride depends on the order of stimulus and drug application. However, Hettinger and Frank (13) noted that, for NaCl stimuli, brief pretreatment of NaCl-best chorda tympani fibers with amiloride inhibited responses as completely as addition of the drug during stimulation. In summary, these results emphasize that there are variable effects of amiloride and its derivatives on whole nerve, single chorda tympani fibers and single NST unit responses to HCl that are presently poorly understood. We speculate that the drug and stimulus actions are governed by physiological state-dependent changes in the TRC at the channel level and perhaps during adaptation in some animals. Changes in channel function could be related to individual differences in degradation and turnover (13) and/or hormonal regulation of apical Na\(^+\) channels (11, 13).

Studies on the effect of amiloride on taste behavior in hamsters suggest that this drug may diminish the aversiveness of NaCl taste (13). However, postigestional effects of amiloride could not be completely ruled out. These studies have been confirmed and extended to include the effect of amiloride on acid-related taste behavior in hamsters (9). The taste aversiveness of pH 2.4 citric acid was diminished when dissolved in 30 µM amiloride, although overall this solution was still rejected. The results were obtained in 96-h, two-bottle preference aversion tests. The lengthy duration of this testing period, however, makes it difficult to distinguish taste-guided behaviors, per se, from ingestive behaviors that result from postabsorptive consequences of amiloride and citric acid intake.

**Tracking of pH by Hamster TRCs**

Steady-state TRC pH\(_i\). In control solutions (pH\(_o\) = 7.4), the mean resting TRC pH\(_i\) was 7.46 ± 0.04, and, like rat TRCs (18), hamster fungiform TRCs are capable of regulating pH\(_i\) when challenged with weak acids (Fig. 12). Incubation of TBFs in amiloride or in Na\(^+\)-free Ringer solutions (Figs. 9–11) induced small, but significant, decreases in steady-state TRC pH\(_i\), suggesting that some taste cell pH regulatory mechanisms are partially amiloride and Na\(^+\) sensitive. For example, a decrease in pH\(_i\) under such conditions may be attributed to inhibition of an electroneutral, amiloride-sensitive Na\(^+\)/H\(^+\) exchange in the TRC membranes. Alternatively, the inhibition by amiloride of apical Na\(^+\) channels or Na\(^+\) removal in the absence of amiloride could cause hyperpolarization of the cell membrane potential and a consequent potential-
dependent decrease in pHi (17). However, the nature of the pH regulatory pathways in TRCs is not known.

Relationship between pHi and pHo in TRCs. As in earlier work (18), we used two techniques, a photomultiplier tube (PMT) system and an image acquisition system, to monitor pHi. The two systems weigh the data differently. The PMT system yields pHi values on the basis of the total amount of light emitted in the area of study, and all the cells within the area contribute to the pHi in proportion to their BCECF content. The image acquisition system yields pHi values in the spatial domain in which the ROIs are calibrated and analyzed individually. Therefore, PMT studies do not yield information regarding individual cells in the area limited by the field diaphragm. Figures 8–12 present data from PMT studies and yield data from an average of three to five cells. The variation in pHi in individual ROIs in TBFs was investigated by imaging. Figure 6 shows that step changes in pHo induced a wide distribution in pHi values in different ROIs in the TBFs. In 20 ROIs, the S of the relation between pHi and pHo varied between 0.51 and 1.42, with a mean value (±SD) of 1.05 ± 0.22. This distribution demonstrates a heterogeneity in cell pH tracking ability that may correspond to cells with different functions. The variation in the pHi response suggests that it is unlikely that all cells, in which pHi varies with pHo, participate in acid-taste transduction (18). It is possible that acid-induced changes in pHo modulate taste cell responses to other taste stimuli, such as sugars or alkaloids (7, 27). A unit change in pHo caused changes in pHi of ~0.65 pH units in type 1 cells of the carotid body, the primary chemosensors of arterial blood pH (3, 21). Although a linear relationship between pHi and pHo appears to be characteristic of cells that function as pH sensors (21), it is possible that cells that are not acid sensors may also have this property.

Effect of amiloride, benzamil, and Na+ removal on pHo-induced changes in pHi. As shown in Figs. 6–11, microfluorometric and imaging studies revealed that the association between pHi and pHo was rapid and pHo appears to be characteristic of cells that function as pH sensors (21), it is possible that cells that are not acid sensors may also have this property. Since in vitro studies, amiloride and its analogs did not affect pHo-induced changes in pHi (18). In addition, our data show that both chorda tympani responses to HCl and pH tracking by TRCs are independent of Na+/H+ exchange in TRC membranes. Although the in vitro measurements of pHi presented here do not distinguish between H+ flux across the apical and the basolateral membrane of TRCs, results from chorda tympani recordings in hamsters (this study) and in rats (6) suggest that protons that evoke most of the neural response to acids do not traverse an apical conductance. Instead, H+ ions, like K+ (31), may access a basolateral transduction site following diffusion through taste bud tight junctions.

It is interesting that, under our experimental conditions, the ability of TRCs to recover from changes in pHi is also dependent on pHo. Both hamster and rat TRCs recover spontaneously from changes in pHi caused by exposure of the cells to weak acids or bases at iso-pHo. In contrast, when changes in pHi were caused by alteration of pHo, TRCs did not spontaneously recover their pHi but instead tracked pHo monotonically. Because TRC pHi was a function of external HCO3 concentration at constant PCO2 (18), it sug-
gested that, under physiological conditions, salivary HCO$_3^-$ participates in the maintenance of steady-state TCR pH, and neutralizes acid-induced changes in pH$_i$.

(27)

Overall, our data lead to the following conclusions.
1) The chorda tympani responses of hamster to NaCl are, like rat, predominantly mediated through an amiloride- and voltage-sensitive transduction pathway, most probably apical membrane epithelial Na$^+$ channels.
2) The transduction pathways that yield chorda tympani responses to HCl in hamster are, like those of rat, predominantly voltage and amiloride insensitive.
3) Isolated taste cells in hamster, like those of rat, have cellular mechanisms that correct perturbations in pH$_i$ when they are made at constant extracellular pH.
4) However, a significant fraction of cells rapidly changes pH$_i$ in proportion to changes made in pH$_o$, i.e., they track pH. 5) pH tracking may be a component of the cellular transduction mechanism in H$^+$ taste sensing, analogous to H$^+$-sensing mechanisms proposed for carotid body chemoreceptor cells. This last point should be regarded as a fresh source of testable hypotheses aimed at elucidating cellular events in H$^+$ taste sensing.

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