Na\textsuperscript{+}-H\textsuperscript{+} EXCHANGE ACTIVITY IN TASTE RECEPTOR CELLS

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ABSTRACT

Messenger RNA for two Na\(^+\)-H\(^+\)-exchanger isoforms 1 and 3 (NHE-1 and NHE-3) was detected by RT-PCR in fungiform and circumvallate taste receptor cells (TRCs). Anti-NHE-1 antibody binding was localized to the basolateral membranes, and the anti-NHE-3 antibody was localized in the apical membranes of fungiform and circumvallate TRCs. In a subset of TRCs, NHE-3 immunoreactivity was also detected in the intracellular compartment. For functional studies, an isolated lingual epithelium containing a single fungiform papilla was mounted with apical and basolateral sides isolated and perfused with nominally CO\(_2\)/HCO\(_3\)-free physiological media (pH 7.4). The TRCs were monitored for changes in intracellular pH (pH\(_i\)) and Na\(^+\) ([Na\(^+\)]\(_i\)) using fluorescence ratio imaging. At constant external pH: (i) Removal of basolateral Na\(^+\) reversibly decreased pH\(_i\) and [Na\(^+\)]\(_i\). (ii) HOE642, a specific blocker, and amiloride, a non-specific blocker of basolateral NHE-1, attenuated the decrease in pH\(_i\) and [Na\(^+\)]\(_i\). (iii) Exposure of TRCs to basolateral NH\(_4\)Cl or sodium acetate pulses induced transient decreases in pH\(_i\) that recovered spontaneously to baseline. (iv) pH\(_i\) recovery was inhibited by basolateral amiloride, 5-(N-methyl-N-isobutyl)-amiloride (MIA), 5-(N-ethyl-N-isopropyl)-amiloride (EIPA), HOE642, and by Na\(^+\) removal. (v) HOE642, MIA, EIPA, and amiloride inhibited pH\(_i\) recovery with K\(_i\) values of 0.23, 0.46, 0.84, and 29 \(\mu\)M, respectively. (vi) A decrease in apical or basolateral pH acidified TRC pH\(_i\) and inhibited spontaneous pH\(_i\) recovery. The results indicate the presence of a functional NHE-1 in the
basolateral membranes of TRCs. We hypothesize that NHE-1 is involved in sour taste transduction since its activity is modulated during acid stimulation.

**Key Words:** Sour taste, Intracellular pH, intrinsic buffering capacity, fungiform papilla.
INTRODUCTION

Sour taste is elicited by a variety of acidic stimuli that interact with a subset of taste receptor cells (TRCs) in the lingual epithelium (DeSimone et al. 2001a). TRCs are polarized neuro-epithelial cells that can be studied in vitro while maintaining their natural polarity in the lingual epithelium. Stimulating the apical membranes of TRCs with acids induced sustained decreases in intracellular pH (pH$_i$) (Lyall et al. 2001, 2002a, 2002b). During acid-stimulation a decrease in TRC pH$_i$, rather than a decrease in extracellular pH (pH$_e$), is the stimulus intensity variable that correlates specifically with increased chorda tympani (CT) taste nerve activity. Inhibiting acid-induced TRC acidification also inhibits the acid-evoked CT response (Lyall et al. 2001, 2002b). These results indicate that a decrease in TRC pH$_i$ is the proximate stimulus for sour taste.

Further studies indicate that in the case of strong mineral acids (HCl), both apical H$^+$ entry and H$^+$ exit across the basolateral membrane of TRCs are regulated by second messengers, which also modulate CT responses to HCl (Lyall et al. 2002a). An increase in intracellular adenosine 3',5'-cyclic-monophosphate (cAMP) enhanced the sour taste of strong acids by activating a Ca$^{2+}$- and amiloride-insensitive apical H$^+$ conductance and inhibited pH$_i$ recovery in TRC membranes. In contrast, an increase in intracellular Ca$^{2+}$ concentration ([Ca$^{2+}]_i$) stimulated pH$_i$ recovery in TRCs, which increased sensory adaptation to acids (Lyall et al. 2002a). Overall, the data indicate that pH recovery mechanisms in TRC membranes play an important role in sour taste transduction and adaptation, but these have not been characterized so far.
Isolated rat (Lyall et al. 1997) and hamster (Stewart et al. 1998) TRCs, maintained at pH\(_o\) of 7.4, demonstrated spontaneous pH\(_i\) recovery from intracellular acid loading. However, during acid stimulation, i.e. when changes in TRC pH\(_i\) were induced by a decrease in pH\(_o\), no spontaneous recovery of TRC pH\(_i\) was observed. Together these results suggest that TRCs regulate pH\(_i\) when perturbations in pH\(_i\) occur at constant pH\(_o\) of 7.4, but pH regulatory mechanisms are attenuated during acid stimulation (i.e. when pH\(_o\) is decreased). Because TRCs are epithelial cells, with distinct apical and basolateral membrane domains, pH-regulatory mechanisms can differ in kind and function between membrane domains (Josette and Pouyéssgur 1995; Ritter et al. 2001). We have, therefore, used a method that allows us to make measurements on a single fungiform taste bud while maintaining a normal polarized epithelial environment (Lyall et al. 2001, 2002a, 2002b; Simon 2002). Using pH imaging, Na\(^+\) imaging, RT-PCR, and immunocytochemical methods, we have identified both Na\(^+\)-H\(^+\) exchanger isoform 1 (NHE-1) and isoform 3 (NHE-3) in fungiform and circumvallate TRCs. We present evidence that NHE-3 is present in the apical membranes of fungiform TRCs, but it is quiescent under the experimental conditions examined so far. A functional NHE-1 activity is present in the basolateral membrane of TRCs in the fungiform papillae. The functional characteristics of basolateral NHE-1, as they relate to TRC pH\(_i\) regulation and its suggested role in sour taste transduction are presented below.

Preliminary reports of this work have been published in abstract form (Lyall et al. 2000a; Vinnikova et al. 2001).
MATERIALS AND METHODS

**RT-PCR.** Rat fungiform and circumvallate taste buds were isolated by collagenase treatment (Béhé et al. 1990). Taste buds were aspirated with a micropipette (diameter ~100 µm) from either fungiform papillae in the anterior tongue or from the circumvallate papilla in the posterior tongue. About 100 taste buds (pooled from 3 rats) from each region of the tongue were individually transferred onto cover slips, avoiding contaminating cells and debris. Total RNA was extracted using the RNeasy Mini kit (Qiagen) according to the manufacturer’s instructions. RT-PCR was performed with a One-Step RT-PCR Kit (Qiagen) using NHE-1 and NHE-3 primer sequences and PCR conditions described elsewhere (Borensztein et al. 1995). The PCR products were analyzed by direct sequencing (Davis Sequencing, CA).

**Immunocytochemistry.** Rats were anesthetized with isoflurane and perfused via the left ventricle with phosphate buffered saline (PBS) followed by 2% paraformaldehyde solution in PBS for 10 minutes. Tongues and kidneys were harvested, embedded in paraffin and sectioned. The immunocytochemical procedure was performed as described previously (Hager et al. 2001) with some modifications. Briefly, 5 µm sections were deparaffinized, re-hydrated and subjected to antigen unmasking using High pH Target Retrieval Solution (Dako) (for NHE-3) or 1% sodium dodecyl sulfate (for NHE-1). Following the blocking procedure, sections were incubated overnight at 4 °C with primary antibodies. The monoclonal anti-NHE-1 antibodies were obtained from Chemicon. The
polyclonal NHE-3 antibodies were generated in Dr. Mark Knepper’s laboratory, and have been characterized previously (Fernandez-Llama et al. 1998; Kim et al. 1999). The monoclonal anti-Na\(^+\)-K\(^+\)-ATPase antibodies were obtained from Upstate Biotechnology and used as a marker of the basolateral membrane. NHE-1 and Na\(^+\)-K\(^+\)-ATPase immunoreactivity was detected using Alexa-488 labeled goat-anti-mouse IgG (Molecular Probes). NHE-3 immunoreactivity was detected using the multi-step immunoperoxidase technique with biotinylated anti-rabbit IgG followed by Cy3-labelled antiperoxidase antibodies (Jackson ImmunoResearch Laboratories). Alexa-488 and Cy3 fluoroprobes have non-overlapping spectra and were used in dual-labeling experiments. Sections were mounted in ProLong Antifade medium (Molecular Probes) and were imaged using a Nikon laser scanning confocal microscope. The images were analyzed using Photoshop software.

**pH and Na\(^+\) imaging.** Rats were anesthetized with isoflurane and killed by cervical dislocation. The tongues were rapidly removed and stored in ice-cold Ringer’s solution pH 7.4 (R; Table 1). The lingual epithelium was isolated by collagenase treatment (Lyall et al. 1997; Stewart et al. 1998). A small piece of the anterior lingual epithelium containing a single fungiform papilla was mounted in a special microscopy chamber (Chu et al. 1995) as described before (Lyall et al. 2001, 2002a, 2002b; DeSimone et al. 2001b). For the measurement of pH\(_i\), TRCs within the taste bud were loaded with BCECF and for Na\(^+\) measurement ([Na\(^+\)]\(_i\)) the taste cells were loaded with either SBFI or Na-green. The detailed
methods for the measurement of pH, using BCECF (Lyall et al. 2001, 2002a, 2002b; DeSimone et al. 2001b) and the measurement of [Na⁺]ᵢ using SBFI or Na-green have been described earlier (Lyall et al. 2002b). In brief, TRCs in the taste bud were visualized from the basolateral side through a 40x objective (Zeiss; 0.9 NA) with a Zeiss Axioskop 2 plus upright fluorescence microscope and imaged with a set up consisting of: a cooled CCD camera (Imago, TILL Photonics, Applied Scientific Instrumentation, Eugene, OR) attached to an image intensifier (VS4-1845 Videoscope, Washington, DC), an epifluorescent light source (TILL Photonics Polychrome IV), dichroic beam splitters, and emission filters for BCECF and fura-2 (Omega Optical). Small regions of interest (ROIs) within the taste bud (diameter 2-3 µm²) were chosen in which the changes in fluorescence of the single wavelength dye (sodium green) or the FIR (fluorescence intensity ratio) for a dual excitation dye (BCECF or SBFI) were analyzed using TILLvisION v3.1 imaging software. Each ROI contained 2-3 receptor cells. Thus the fluorescence intensity recorded for a ROI represents the mean value from 2-3 receptor cells within the ROI. In a typical experiment, the FIR measurements were made in an optical plane in the taste bud containing 4-6 ROIs (approximately 12-18 cells). The background and autofluorescence were corrected from images of a taste bud without the dye. Amiloride, 5-(N-methyl-N-isobutyl)-amiloride (MIA), 5-(N-ethyl-N-isopropyl)-amiloride (EIPA), and cariporide (HOE642) exhibit strong U.V. fluorescence at 380 nm. For this reason, SBFI is not suitable for measuring changes in TRC [Na⁺]ᵢ in the presence of
these drugs. Accordingly, these experiments were performed with the single wavelength excitation dye, Na-green (Lyall et al. 2002b).

**Solutions.** The composition of the solutions used in the *in vitro* experiments is given in Tables 1 and 2. The control solution was Ringer’s solution without Na-pyruvate (RC; Table 1) (Lyall et al. 2002a, 2002b). In Na\(^+\)-free solutions, NaCl was replaced with an equivalent amount of N-methyl-D-glucamine chloride (NMDGCl; R0Na; Table 1). Some solutions contained amiloride, MIA, EIPA (Sigma), the non-specific blockers or HOE642 (Aventis Pharma, Germany), a specific blocker of the NHE-1 (Scholz et al. 1995). In some experiments, the apical membrane was perfused with a solution without HEPES containing 58.3 mM acetic acid (RAA; pH 3.0; Table 1) or 1 mM HCl (pH 3.0). For the measurement of intrinsic buffering capacity (\(\beta_1\)) we used Na\(^+\)- and Cl\(^-\)-free solutions (R0Na0Cl; Table 2). To block NH\(_4^+\) flux via the K\(^+\) channels, 10 mM tetraethylammonium acetate (TEAA) was added to the solutions. In these experiments, the apical solutions also contained, in addition, 2 mM cetylpyridinium chloride (CPC) to block the NH\(_4^+\) flux via the amiloride-insensitive CPC-sensitive cation channels in the apical membranes of fungiform TRCs (DeSimone et al. 2001b). Different concentrations of NH\(_4^+\) were obtained by mixing the R0Na0Cl and R(NH\(_4\))\(_2\)SO\(_4\) (Table 2) solutions. At the end of each experiment, TRC pH\(_i\) was calibrated using the calibrating solutions (CS; pH 6.5-8.0; Table 2) containing 10 \(\mu\)M nigericin.
**Data analysis.** The changes in TRC pH$_i$ were expressed as the mean ± standard error of the mean of n; where n represents the number of ROIs within the taste bud; M ± SEM (n). In TRCs loaded with sodium-green the changes in [Na$^+$]$_i$ were expressed relative to the fluorescence intensity ($F_{490}$) under control conditions. The $F_{490}$ under control conditions for each ROI was taken as 100%. For TRCs loaded with SBFI, the relative changes in FIR ($F_{340}/F_{380}$) were compared between individual ROIs under different experimental conditions. The data were also presented as the mean ± standard error of the mean from different tissue preparations (N). In this case N represented the number of individual polarized lingual preparations studied. Student’s t-test was employed to analyze the differences between sets of data.
RESULTS

We have previously shown that at a constant pH$_o$ of 7.4, following intracellular acid loading with weak organic acids, both isolated (Lyall et al. 1997; Stewart et al. 1998) and polarized (Lyall et al. 2002a, 2002b) TRCs spontaneously recovered their pH$_i$ to resting levels. This suggests that the TRC membranes must contain pH compensatory mechanisms that spontaneously restore pH$_i$ to resting levels. We, therefore, hypothesized that the most common epithelial isoforms of NHE, namely apical NHE-3 and basolateral NHE-1, would be present and functional in TRCs. In this study, we first used RT-PCR to detect the presence of NHE mRNA transcripts in fungiform and circumvallate TRCs. Secondly, we used specific antibodies to localize the presence of NHEs in TRCs membranes, and lastly, we used pH and Na$^+$ imaging to characterize the functional role of NHEs in TRC pH$_i$ regulation.

Presence of NHE-1 and NHE-3 mRNA in TRCs. Figure 1 shows RT-PCR in fungiform taste buds for NHE-1 in lanes 2-5. Using specific primers for NHE-1 (Borensztein et al. 1995), a 422 bp product of expected size was observed in taste buds (lane 2) and in positive kidney control (lane 4). Figure 1 also shows the RT-PCR for NHE-3 mRNA in lanes 7-10. Using specific primers for NHE-3 (Borensztein et al. 1995), a 321 bp product of expected size and a larger size genomic band was seen (lane 7). In taste buds, in which the reverse transcription step was omitted, only the genomic band was seen (lane 8). In kidney positive control (lane 9), a single 321 bp product was observed (genomic DNA was not
amplified). The negative control without template is shown in lane 10. Identity of the PCR products as rat NHE-1 and NHE-3 was confirmed by sequencing. In parallel experiments, we were also able to confirm the presence of NHE-1 and NHE-3 mRNAs in circumvallate taste buds (data not shown).

**Presence of NHE-1 and NHE-3 in TRC membranes.** Using specific NHE-3 antibodies and confocal imaging, NHE-3 was found to be present in the apical region (arrow) of the fungiform (FF) TRCs (Fig. 2B; red label). The kidney (K) control (Fig. 2A; red label) demonstrated the specific binding of the NHE-3 antibodies to the brush border of the proximal tubules. The green label shows the basolateral Na\(^+\)-K\(^+\)-ATPase. In some sections of fungiform papillae, the NHE-3 was also localized in the intracellular compartment (Fig. 2C; red label). The NHE-3 label was not present in the basolateral membranes of TRCs since NHE-3 did not co-localize with the basolateral Na\(^+\)-K\(^+\)-ATPase (Fig. 2C; green label).

In fungiform (FF) papilla, the NHE-1 antibody binding was localized to the basolateral membranes of TRCs (Fig. 3A; green label). The kidney (K) control (Fig. 3B; green label) demonstrated specific binding of NHE-1 antibodies to the basolateral membranes of kidney collecting tubules. Figures 3C and 3D show the localization of NHE-1 and NHE-3 antibody binding in the circumvallate (CV) papilla. Similar to the case with fungiform (FF) taste buds, the NHE-3 antibody binding was localized to the apical membranes (arrows) of circumvallate TRCs (Fig. 3D; red label) and the NHE-1 antibody binding was localized to the basolateral membranes of circumvallate TRCs (Fig. 3C; green label). These
results indicate that similar to other transporting epithelia, the apical and basolateral membranes of TRCs contain NHE-3 and NHE-1 isoforms, respectively. In the next series of experiments we determined if NHE-1 and NHE-3 are involved in pH$_i$ regulation in TRCs.

**Effect of external Na$^+$ on TRC pH$_i$.** In polarized TRCs initially perfused on both sides with control Ringer’s solution (RC; Table 1), switching to a Na$^+$-free solution (R0Na; Table 1) in the apical compartment (Fig. 4A, a-b) did not alter resting TRC pH$_i$. In contrast, switching to R0Na in the basolateral compartment (Fig. 4A, b-c) decreased the mean TRC pH$_i$ from 7.26 ± 0.03 to 6.70 ± 0.03 (n = 6). Re-perfusing the control Ringer’s solution (RC) in the basolateral compartment (c-d) promptly increased pH$_i$ to its original level. In three preparations, no changes in TRC resting pH$_i$ were observed upon perfusing Na$^+$-free solution in the apical compartment. In contrast, perfusing the Na$^+$-free solution in the basolateral compartment decreased resting TRC pH$_i$ by 0.62 ± 0.03 pH unit (p< 0.001; N = 3). These results indicate that TRC pH$_i$ is dependent upon the Na$^+$ concentration in the basolateral compartment, but is independent of apical Na$^+$ concentration.

**Relationship between basolateral Na$^+$ concentration and TRC pH$_i$**

The TRC pH$_i$ was monitored during step changes in basolateral NaCl concentration from 0 to 1, 2.5, 5, 10, 20, 50, 100 and 150 mM. Varying concentrations of Na$^+$ were achieved by mixing RC and R0Na in appropriate proportions (Table 1). The data in Fig. 5A show that in a polarized TRC
preparation perfused on both sides with Na\(^+\)-free solution (R0Na; Table 1), successively increasing the basolateral Na\(^+\) concentration from 0 to 150 mM, increased TRC pH\(\text{r}_i\) in a stepwise manner. The steady-state pH\(\text{r}_i\) values at each successive Na\(^+\) concentration are plotted in Fig. 5B. The curve was drawn according to a kinetic model of NHE activity (cf. Appendix). In the complete absence of Na\(^+\) the mean pH\(\text{r}_i\) was 6.62 ± 0.003 (n = 4). At a basolateral Na\(^+\) concentration of 50 mM, the pH\(\text{r}_i\) increased to 7.47 ± 0.005. Increasing the Na\(^+\) concentration from 50 mM to 100 mM, and then to 150 mM increased pH\(\text{r}_i\) to 7.61 ± 0.005 and 7.65 ± 0.005, respectively. These results indicate that increasing the basolateral Na\(^+\) concentration from 0 to 50 mM increased pH\(\text{r}_i\) to 82.7% of its resting value under control conditions.

The initial rate (measured for the first 100 s) at which pH\(\text{r}_i\) acidified was also dependent upon the imposed Na\(^+\) gradient across the basolateral membrane of TRCs. In a polarized TRC preparation, the mean initial acidification rate (δpH\(\text{r}_i\)/min) achieved its maximum value (−0.17 pH unit/min) during a step change in the basolateral Na\(^+\) concentration from 150 mM to 0 and decreased to −0.03 pH unit/min during a step change in Na\(^+\) concentration from 150 mM to 50 mM. Small, but significant changes in pH\(\text{r}_i\) occur when the basolateral Na\(^+\) concentration is varied from 50 to 150 mM (Figs. 5A and 5B), suggesting that the overall resting TRC pH\(\text{r}_i\) is determined by several Na\(^+\)-dependent pH regulatory mechanisms in TRC membranes.

These results suggest that a Na\(^+\)-dependent acid extrusion mechanism is present in the basolateral membranes of fungiform TRCs. In the apical
membranes of TRCs a Na\(^+\)-dependent acid extrusion mechanism seems to be either absent or is quiescent under our experimental conditions. To test the possibility that this mechanism is a Na\(^+\)-H\(^+\) exchanger, further experiments were performed in the presence of basolateral NHE blockers, amiloride, MIA, EIPA, and HOE642 (Scholz et al. 1995).

**Effect of NHE blockers on TRC pH\(_i\).** The data presented in Fig. 4A also show that perfusing the basolateral membrane of polarized TRCs with control Ringer’s solution (RC; Table 1) containing 1 µM HOE642, a specific blocker of the NHE-1, decreased resting TRC pH\(_i\) (d-e) from 7.26 ± 0.01 to 7.15 ± 0.03 (ΔpH\(_i\) = -0.11 ± 0.002; p<0.01; n = 6). In the continuous presence of HOE642, lowering the basolateral NaCl concentration from 150 mM to 0 (switching to R0Na + 1 µM HOE642; Table 1) decreased TRC pH\(_i\) from 7.15 ± 0.03 to 6.91 ± 0.02. Thus in the presence of the 1 µM HOE642 the magnitude of TRC pH\(_i\) (ΔpH\(_i\) = -0.24; e-f) was decreased by approximately 59% relative to control (ΔpH\(_i\) = -0.58; b-c). The initial rates of changes in TRC pH\(_i\) were measured for the first 100 s following a change in basolateral Na\(^+\) concentration. In the absence of HOE642, decreasing the basolateral Na\(^+\) concentration from 150 mM to 0 decreased TRC pH\(_i\) at the mean rate of –0.065 ± 0.009 pH unit/min (Fig. 4A; b-c) and upon re-perfusing with control Ringer’s solution containing 150 mM NaCl increased pH\(_i\) at the mean rate of 0.227 ± 0.017 pH unit/min (Fig. 4A; c-d; n = 6). In contrast, in the presence of HOE642, the corresponding mean rates of changes in pH\(_i\) upon Na\(^+\) removal (e-f) and re-addition (f-g) were –0.021 ± 0.003 (67.7% inhibition) and
0.014 ± 0.003 pH unit/min (93.8% inhibition), respectively. Upon perfusing control Ringer’s solution (RC) without HOE642, promptly increased TRC pH_i to its resting level (g-h). In the final step, re-perfusing the control Ringer’s solution (RC) in the apical compartment did not produce any further changes in TRC pH_i (h-i). In additional experiments, in polarized TRCs perfused on both sides with the Na^+-free solution, perfusing the control Ringer’s solution in the apical compartment also had no effect on TRC pH_i (data not shown). In an additional experiment, perfusing the basolateral membrane of polarized TRCs with Na^+-free Ringer’s solution, decreased TRC pH_i from 7.30 ± 0.01 to 6.79 ± 0.01 (Fig. 4B; j-k-l; mean ΔpH_i = 0.51). And in the presence of 10 µM HOE642, perfusing 0 Na^+ Ringer’s solution in the basolateral compartment, decreased TRC pH_i from 7.21 ± 0.01 to 7.10 ± 0.01 (Fig. 4B; m-n; mean ΔpH_i = 0.11). Thus in the presence of 10 µM HOE642, the magnitude of TRC pH_i was decreased by approximately 78.4 % relative to control.

Similar effects were also observed with amiloride, a non-specific blocker of the NHEs. In the absence of amiloride, decreasing basolateral Na^+ concentration from 150 mM (RC) to 0 (R0Na) decreased the mean resting TRC pH_i from 7.23 ± 0.02 to 6.55 ± 0.01 at a rate of 0.108 ± 0.002 pH unit/min (n = 5). Upon reintroducing 150 mM NaCl solution in the basolateral compartment, pH_i increased at a rate of 0.337 ± 0.004 pH unit/min. In the presence of basolateral amiloride (1 mM) the resting TRC pH_i decreased from 7.30 ± 0.01 to 7.26 ± 0.01 (ΔpH_i = -0.04 ± 0.002; p < 0.001, n = 6). In the continuous presence of amiloride, lowering basolateral Na^+ concentration from 150 mM to 0, decreased TRC pH_i at
a mean rate of 0.063 ± 0.004 pH unit/min, around a 40% inhibition. Reintroducing 150 mM NaCl solution in the basolateral compartment, increased pH$_i$ at a rate of 0.029 ± 0.002 pH unit/min, around a 90% inhibition. These rates are significantly less than the corresponding rates in the absence of amiloride (p < 0.001; paired; n = 5). Taken together the data suggest the presence of a HOE642-sensitive Na$^+$$\text{-}$H$^+$ exchange mechanism in the basolateral membranes of TRCs. In the next series of experiments we investigated the involvement of this exchanger in the regulation of TRC pH$_i$.

**Effect of intracellular acid loading on TRC pH$_i$**

To investigate if the basolateral Na$^+$$\text{-}$H$^+$ exchange activity is involved in TRC pH$_i$ regulation, we monitored the rate of spontaneous pH$_i$ recovery following intracellular acid loading with NH$_4$Cl or Na-acetate at constant pH$_o$ (Lyall *et. al.* 2002a, 2002b).

**Studies with NH$_4$Cl.** Figure 6 shows the effect of a short basolateral NH$_4$Cl pulse on TRC pH$_i$. Immediately following NH$_4$Cl perfusion (RNH$_4$Cl; Table 1), TRC pH$_i$ rapidly alkalinized (a-b), presumably, due to the entry of NH$_3$ and the conversion of free intracellular H$^+$ ions to NH$_4^+$ ions (Roos and Boron 1981). This was followed by a slow decline of pH$_i$ towards baseline (b-c), presumably, reflecting NH$_4^+$ entry or pH compensation mechanism(s) in TRC membranes (Roos and Boron 1981). Upon replacing NH$_4$Cl solution with a Na$^+$-free solution (R0Na; Table 1) in the basolateral compartment, TRC pH$_i$ acidified (c-d), and
became lower than its resting value due to the combined effect of rapid NH$_3$ exit from the cells, the conversion of NH$_4^+$ to NH$_3$ + free H$^+$ ions, and the removal of basolateral Na$^+$ (cf. Fig. 4). No spontaneous recovery of pH$_i$ was observed in the absence of basolateral Na$^+$ (Fig. 6; d-e). Perfusing the basolateral compartment with control Ringer’s solution (RC; Table 1), promptly increased TRC pH$_i$ to its resting value (Fig. 6; e-f). The spontaneous pH$_i$ recovery rate following an NH$_4$Cl pulse was dependent upon the basolateral Na$^+$ concentration (Fig. 7). Increasing the Na$^+$ concentration, in a stepwise manner, from 0 to 150 mM, caused the spontaneous pH$_i$ recovery rate to increase as a saturating function of [Na$^+$]$_{Bl}$ ($K_m = 8.3$ mM). This is consistent with the predicted kinetics of NHE (Grinstein and Rothstein 1986; Appendix).

The data summarized in Fig. 6 also show that following a second NH$_4$Cl pulse, replacing NH$_4$Cl solution (RNH$_4$Cl) with control Ringer’s solution (RC; Table 1) containing 1 $\mu$M HOE642 in the basolateral compartment, TRC pH$_i$ acidified and became lower than its resting value (f-g). This decrease in pH$_i$ was transient and slowly recovered towards baseline (g-h) at a rate of $0.01 \pm 0.001$ pH unit/min. Perfusing the basolateral membrane with control Ringer’s solution (RC; Table 1) without the drug, increased the spontaneous rate of pH$_i$ recovery to $0.048 \pm 0.003$ pH unit/min (h-i). This value was not different from the pH$_i$ recovery rate ($0.047 \pm 0.001$ pH unit/min) in control Ringer’s solution without HOE642 (j-k). These results indicate that at constant pH$_o$, pH$_i$ recovery from an acid load is dependent upon the basolateral Na$^+$ concentration and is inhibited by HOE642.
HOE642 inhibited the spontaneous rate of pH$_i$ recovery after the NH$_4$Cl pulse in a dose dependent manner (Fig. 8; open circles and dotted line). The data were fit to a Michaelis-Menten type equation. In 3 independent polarized TRC preparations, the mean K$_i$ value (the concentration that inhibits the spontaneous rate of pH$_i$ recovery by 50%) for HOE642 was 0.23 $\mu$M (range = 0.14 to 0.40 $\mu$M). In separate experiments, we also tested the inhibition of NHE-1 activity by MIA (filled triangles and dash-dot line), EIPA (filled squares and short dash line), and amiloride (filled circles and solid line). In 3 taste buds, the mean K$_i$ for HOE642, MIA, EIPA, and amiloride was 0.23, 0.46, 0.84, and 29 $\mu$M, respectively (Fig. 8).

**Studies with Na-acetate.** Figure 9 shows the effect of a short basolateral side Na-acetate (NaA) pulse on TRC pH$_i$. Consistent with our previous observations (Lyall et al. 2002b), immediately following the perfusion of Ringer’s solution containing 30 mM NaA (RNA; Table 1) in the basolateral compartment pH$_i$ rapidly acidified (a-b), presumably due to the entry of membrane-permeable undissociated acetic acid and its conversion to free intracellular H$^+$ ions plus acetate anion (Roos and Boron 1981). The intracellular acidification was transient and was followed by a spontaneous recovery of pH$_i$ to baseline (b-c). Upon Na-acetate washout (RC; Table 1), TRC pH$_i$ alkalinized and became higher than its resting value (c-d). This is due to the rapid exit of the undissociated acetic acid from cells and a decrease in intracellular H$^+$ ions. The spontaneous recovery of alkaline pH$_i$ towards baseline (d-e) reflects the presence of as yet an unknown
pH recovery mechanism in TRCs that allows base (OH\(^{-}\)) exit or entry of acid equivalents at alkaline pH\(_i\). In the presence of 10 µM HOE642, the spontaneous mean initial pH\(_i\) recovery rate (f-g; 0.009 ± 0.003 pH units/min) was significantly reduced (81.2% inhibition) compared to control (b-c; 0.048 ± 0.004 pH units/min; n = 5). However, HOE642 did not affect the exit of the undissociated acetic acid from cells (g-h) and the subsequent spontaneous recovery of alkaline pH\(_i\) towards baseline (h-i). Similar results were obtained in 2 additional experiments (data not shown). The results indicate that HOE642 specifically inhibits pH\(_i\) recovery from an intracellular acid load and does not affect mechanisms involved in base (OH\(^{-}\)) exit or entry of acid equivalents at alkaline pH\(_i\). The results further suggest that the basolateral NHE-1 is involved in the regulation of TRC pH\(_i\).

However, for the highest concentrations of the NHE-1 blockers: MIA, EIPA, HOE642, and amiloride, the mean pH\(_i\) recovery from an NH\(_4\)Cl pulse was maximally inhibited by 74, 95, 70, and 80% (Fig. 8). Consistent with this, in Fig. 9, 10 µM HOE642 inhibited pH\(_i\) recovery from Na-acetate pulse by 81.2%. In addition, 10 µM HOE642 inhibited the magnitude of TRC pH\(_i\) decrease induced by basolateral Na\(^+\) removal by approximately 78% (Fig. 4B; j-k-l). Thus none of the dose response curves achieved the 100% inhibition level for high doses of the drug. These data indicate that basolateral NHE-1 is the major pH regulatory mechanism in TRCs, and accounts for about 80% of the pH\(_i\) regulation in TRCs. However, NHE-1 is not the only pH regulatory mechanism in TRCs. It is likely that about 20% of the remaining pH\(_i\) regulation occurs via pH\(_i\) regulatory mechanisms not blocked by HOE642, MIA, EIPA, and amiloride.
Relationship between TRC pH\textsubscript{i} and [Na\textsuperscript{+}]\textsubscript{i}.

Figure 10A shows the effect of external Na\textsuperscript{+} concentration on the relative changes in TRC [Na\textsuperscript{+}]\textsubscript{i} in a polarized fungiform taste bud preparation loaded with Na-green. Upon lowering the apical Na\textsuperscript{+} concentration from 150 mM (RC) to 0 (R0Na), there was a decreased resting F\textsubscript{490} (a-b), indicating a decrease in TRC [Na\textsuperscript{+}]\textsubscript{i}. In the second step, lowering the basolateral Na\textsuperscript{+} concentration from 150 mM to 0, further decreased F\textsubscript{490} (b-c). These changes in TRC [Na\textsuperscript{+}]\textsubscript{i} were reversed upon addition of external Na\textsuperscript{+} to the basolateral (c-d) and apical (d-e) compartments, respectively. The fluorescence of single wavelength dyes can be affected by changes in cell volume, dye leakage, dye bleaching, and changes in focus due to tissue movement. Measurements using a ratiometric dye are not affected by the above factors. Therefore, we also performed some experiments with the dual excitation, single emission dye, SBFI. In polarized TRCs loaded with SBFI the temporal changes in [Na\textsuperscript{+}]\textsubscript{i} were measured as changes in FIR (F\textsubscript{340}/F\textsubscript{380}). Consistent with the data shown in Fig. 10A, a decrease in Na\textsuperscript{+} concentration from 150 mM (RC) to 0 (R0Na; Table 1) in the basolateral compartment decreased FIR (Fig. 10B; a-b), indicating a decrease in TRC [Na\textsuperscript{+}]\textsubscript{i}. Upon re-perfusing the control Ringer's solution (RC; Table 1) in the basolateral compartment, the FIR promptly increased to its original value (b-c).

The above results indicate that the Na\textsuperscript{+} flux occurs across both the apical and basolateral membranes of fungiform TRCs. Comparing Figs. 4A and 10A\textsubscript{z}, further indicates that the apical Na\textsuperscript{+} flux is not coupled to changes in TRC pH\textsubscript{i}.
(Fig. 4A; a-b vs Fig. 10A; a-b). The data suggest that apical NHE-3 is quiescent and does not contribute to the overall Na\(^+\) flux across the apical membranes of TRCs. We have recently (Lyall et al. 2002b) shown that apical Na\(^+\) flux occurs, in part, via the amiloride (or benzamil)-sensitive epithelial Na\(^+\) channels (ENaCs) and by an amiloride (or benzamil)-insensitive pathway, which is modulated by cetylpymridinium chloride (CPC) (DeSimone et al. 2001b).

In contrast, the basolateral Na\(^+\) flux is accompanied by changes in TRC pH\(_i\) (Fig. 4A; b-c vs Fig. 10A; b-c). To investigate if the basolateral Na\(^+\) flux is coupled to changes in TRC pH\(_i\) via the basolateral Na\(^+\)-H\(^+\) exchanger activity, further experiments were done in the presence of amiloride and HOE642. Data summarized in Fig. 11A show that lowering basolateral Na\(^+\) concentration from 150 mM (RC; Table 1) to 0 (R0Na; Table 1) reversibly decreases F\(_{490}\) (a-b-c), and 250 \(\mu\)M amiloride in the basolateral compartment attenuated the changes in F\(_{490}\) induced by Na\(^+\) removal (d-e-f) by 50%. The initial mean rate of change in F\(_{490}\) was measured for the first min following the basolateral Na\(^+\) removal. In three individual taste buds, under control conditions, the F\(_{490}\) declined at the rate of 20.4 \(\pm\) 1.7 %/min and this rate decreased to 6.3 \(\pm\) 1.9 %/min in the presence of 250 \(\mu\)M amiloride (p < 0.01; paired; N = 3; n = 21). Amiloride also inhibited the initial mean rate of increase in F\(_{490}\) induced by changing basolateral Na\(^+\) concentration from 0 to 150 mM by 39.3 \(\pm\) 3.4% (p< 0.01; paired). Similar results were obtained with 10 \(\mu\)M HOE642 (Fig. 11B). As shown in Fig. 4, HOE642 (and amiloride) inhibited changes in TRC pH\(_i\) under identical conditions. Thus both Na\(^+\) and H\(^+\) fluxes across the basolateral membrane are inhibited by HOE642.
and amiloride. Taken together, these results support the presence of a NHE-1 in the basolateral membranes of TRCs, and indicate that a part of the \( \text{Na}^+ \) flux across the basolateral membrane occurs via this mechanism.

**Intrinsic buffering capacity \( (\beta_1) \) of TRCs.** We used the \( \text{pH}_i \) decrease caused by the washout of \( \text{NH}_4^+ \) to compute TRC \( \beta_1 \) (Boyarsky *et al.* 1988; Vaughan-Jones and Wu 1990). Since, at the present time, many of the pH regulatory mechanisms in TRCs have not been characterized, these experiments were done under the conditions in which all acid-base transport mechanisms were inhibited by ion substitution. The experiments were done in nominally \( \text{CO}_2/\text{HCO}_3^- \)-free, \( \text{Na}^+ \)-free, and \( \text{Cl}^- \)-free solutions to block \( \text{Na}^+ \)-, \( \text{Cl}^- \)-, and \( \text{HCO}_3^- \)-dependent pH regulatory mechanisms in TRC cell membranes. The \( \text{NH}_4^+ \) flux via the apical and basolateral \( \text{K}^+ \) channels was blocked by the addition of 10 mM TEAA. The \( \text{NH}_4^+ \) flux via the apical amiloride-insensitive, CPC-sensitive cation pathway was inhibited by the addition of 2 mM CPC in the apical solution (R0Na0Cl; Table 2).

The \( \beta_1 \) (mM/pH unit) was calculated as \( \Delta [\text{H}^+] / \Delta \text{pH}_i \), where \( \Delta [\text{H}^+]_i \) is the amount (in mM) of acid introduced into the cell and \( \Delta \text{pH}_i \) is the resultant change in \( \text{pH}_i \). \( [\text{H}^+]_i \) is assumed to equal the intracellular concentration of \( \text{NH}_4^+ \) \( ([\text{NH}_4^+]_i) \) at the moment of its removal from the external solution (Vaughan-Jones and Wu 1990) and is given by: \( [\text{NH}_4^+]_i = \text{[NH}_4^+\text{]}_o \times 10^{(\text{pHo-}\text{pHi})} \), where \( \text{[NH}_4^+\text{]}_o = C / [10^{(\text{pHo-}\text{pK})} + 1] \). Here \( C \) = the total concentration of external \( \text{NH}_4^+ \), and \( \text{pK} = 9.02 \).

In the example shown in Fig. 12A, lowering total \( \text{NH}_4^+ \)-\( \text{NH}_3 \) from 30 to 20 mM caused the mean TRC \( \text{pH}_i \) to decrease in 16 ROIs within a fungiform taste
bud from 7.51 to 7.38 (ΔpHᵢ = 0.13). We calculate that at 30 mM basolateral NH₄⁺ concentration, the mean [NH₄⁺]ᵢ was 22.4 and, at 20 mM basolateral NH₄⁺ concentration, the mean [NH₄⁺]ᵢ was 20.6 (Δ[NH₄⁺]ᵢ = 1.8). Thus the average β₁ in the small pH range during a change in the basolateral NH₄⁺ concentration from 30 to 20 mM (average pHᵢ 7.44) was (1.8/0.13) = 13.8 mM/pH unit. In a similar manner, subsequently decreasing the NH₄⁺-NH₃ concentration to 10 mM and 5 mM gave mean β₁ value of 24.7 and 26.9 at the average pHᵢ values of 7.28 and 7.10, respectively. The insert in Fig. 12A shows the relationship between mean TRC pHᵢ and β₁ from 3 individual taste buds containing 30 ROIs. The line of best fit (r² = 0.92; n = 30) gave a mean slope of -36.9 (mM/pH)/pH.

In another taste bud, containing 22 ROIs, a step change in basolateral NH₄⁺ concentration from 12 mM to zero, gave a mean β₁ value of 18.7 ± 1.2 mM/pH unit at the mean TRC pHᵢ of 7.38 ± 0.02. In the 22 ROIs studied, the mean resting pHᵢ value varied from 7.27 to 7.61 and the β₁ value ranged between 30.7 and 7.6 mM/pH unit. This indicates that there are significant variations in pHᵢ and β₁ value among individual TRCs within the taste bud. It is likely that TRCs with lower β₁ values respond with a greater decrease in pHᵢ to an acid stimulus and hence, participate most in sour taste transduction. The results further indicate that β₁ increases with a decrease in TRC pHᵢ. The β₁ value was used to calculate the net efflux of acid or the acid-extrusion rate (JᵥH⁺; mM/min) as the product of β₁ and the rate of pHᵢ recovery in TRCs. The β₁ values reported here and their dependence on TRC pHᵢ are comparable to those reported in type 1
carotid body cells, which are involved in CO₂/H⁺ sensing for arterial blood (Buckler et al. 1991a).

In Fig. 12B, the resting pHₖ values under control conditions from 78 ROIs in 7 individual polarized fungiform TRCs preparations were plotted against the number of ROIs that fall within a given pHₖ value. The data demonstrate that two distinct subpopulations of TRCs can be separated based on the initial value of resting pHₖ. The first group demonstrates a normal distribution with a mean pHₖ of around 7.2. The second group demonstrates a skewed distribution with a mean pHₖ around 7.45. Similarly, two subpopulations of TRCs were distinguished by their responses to acid-induced changes in intracellular Ca²⁺ activity (Liu and Simon 2001; Richter et al. 2003). These results further indicate that there are significant variations in resting pHₖ values among individual TRCs within the taste buds. The TRCs having relatively more alkaline resting pHₖ values, and consequently lower β₁ values, will produce larger changes in pHₖ when stimulated by acids. On this basis this cell population might be expected to show higher sensitivity to acids and, therefore, may be the more important cell population for sour taste transduction.

**Effect of pHₙ on the basolateral NHE-1 activity**

Our results (Figs. 6 and 9) indicate that a decrease in TRC pHₖ activates the basolateral NHE-1. To investigate the effect of pHₙ on the basolateral NHE-1 activity, we monitored the spontaneous rate of pHₖ recovery from short NH₄Cl pulses at basolateral pH (pHₙ) of 7.8, 7.4, and 6.8. The pHₖ recovery rate was
measured for the first 2 min following the washout of the NH₄Cl pulse. In a representative experiment (Fig. 13), unilaterally increasing pHᵦ from 7.4 to 7.8, increased pHᵢ from 7.04 ± 0.02 to 7.31 ± 0.02 (e-f) and increased the initial spontaneous pHᵢ recovery rate from 0.060 ± 0.002 (d-e) to 0.076 ± 0.004 (i-j; n = 7). Lowering the basolateral pH from 7.8 to 6.8, decreased pHᵢ from 7.29 ± 0.03 to 6.75 ± 0.01 (j-k) and attenuated the spontaneous rate of pHᵢ recovery from 0.076 ± 0.004 (i-j) to 0.019 ± 0.002 (n-o). In the final step, raising the basolateral pH to 7.4 increased TRC pHᵢ from 6.75 ± 0.01 to 6.96 ± 0.02 (o-p). Using the mean β₁ values of 28.8 and 39.5 (cf. Fig.12A; insert) at the mean TRC pHᵢ values of 7.04 and 6.75, respectively, one can calculate the change in the net mean flux of H⁺ ions (JₜH⁺) at the two pHᵢ values. Thus a decrease in mean TRC pHᵢ from 7.04 to 6.75 (j-k) decreased the mean JₜH⁺ from 1.73 ± 0.06 to 0.75 ± 0.08 mM/min (p< 0.01; n = 7; paired). In three tissue preparations, containing 21 ROIs (N = 3; n = 21), a decrease in basolateral pH from 7.4 to 6.8 decreased the mean resting pHᵢ by 0.38 ± 0.04 (p <0.025) and decreased the mean JₜH⁺ by 47.3 ± 1.4 % (p< 0.001; paired). This decrease in JₜH⁺ represents the decrease in H⁺ flux due to the inhibition of the basolateral NHE-1. The mean data from 4 such experiments are plotted in Fig. 14.

Consistent with our previous studies (Lyall et al. 2001), there was a linear relation between mean steady-state TRC pHᵢ (at points a, f, j, k, and p) and pHᵦ (Fig. 14; filled circles). The line with slope 0.85 is the predicted relation between pHᵢ and pHₒ based on NHE kinetics (see Appendix). NHE kinetics also predicted that the mean spontaneous rate of pHᵢ recovery is an increasing nonlinear
function of pH\textsubscript{Bi}. The mean rates of recovery represented by d-e, i-j, and n-o from 4 experiments are plotted in Fig. 14 as a function of pH\textsubscript{Bi} (open circles). The curve is a least squares fit of the data according to the model developed in the Appendix. The results indicate that unlike changes in TRC pH\textsubscript{i}, changes in pH\textsubscript{o} have an opposite effect on the NHE-1 activity. External acidification inhibits, while external alkalinization, increases the activity of basolateral NHE-1 activity.

Data presented in Fig. 15 show that lowering the apical pH (pH\textsubscript{Ap}) from 7.4 to 3.0 (with acetic acid) decreased resting TRC pH\textsubscript{i} (a-b) from 7.20 ± 0.03 to 6.96 ± 0.03 (\(\Delta\)pH\textsubscript{i} = -0.24 ± 0.007 pH unit; p< 0.001; n = 5). In the presence of apical acetic acid, the spontaneous initial pH\textsubscript{i} recovery rate (measured during the first 100s) from an NH\textsubscript{4}Cl pulse was 0.025 ± 0.003 (\(\Delta\)pH/min; c-d). Returning the pH\textsubscript{Ap} back to 7.4 increased TRC pH\textsubscript{i} (d-e) from 6.87 ± 0.03 to 7.04 ± 0.03 (\(\Delta\)pH\textsubscript{i} = 0.17 ± 0.003 pH unit; p< 0.001; n = 5), and increased pH\textsubscript{i} recovery rate (\(\Delta\)pH/min; f-g) to 0.041 ± 0.002. Thus decreasing apical pH from 7.4 to 3.0 inhibited the initial pH\textsubscript{i} recovery rate by 39.0 ± 5.4% (p< 0.01; paired; n = 5). Using the mean \(\beta\textsubscript{1}\) values of 35.1 and 28.8 (Fig.12A; insert) at the mean TRC pH\textsubscript{i} of 6.91 and 7.04, respectively, one can calculate the change in the net mean flux of H\textsuperscript{+} ions (\(J\textsubscript{H+}\)) at the two pH\textsubscript{i} values. Thus in the presence of apical acetic acid (pH\textsubscript{i} = 6.87 ± 0.03) the mean \(J\textsubscript{H+}\) was 0.88 ± 0.10 mM/min. Upon acetic acid washout, the mean pH\textsubscript{i} increased to 7.04 ± 0.03 and the mean \(J\textsubscript{H+}\) increased to 1.20 ± 0.05 mM/min (p< 0.05; paired; n= 5). In 3 polarized fungiform taste bud preparations, containing 22 ROIs, stimulating the apical membrane with acetic acid (pH 3) produced variable responses in individual ROIs (Lyall et al. 2001).
The acetic acid-induced decrease in pH\textsubscript{i} ranged from 0.05 to 0.30 pH unit with a mean of 0.17 \(\pm\) 0.015 (mean \(\pm\) SEM; n = 22). In the same ROIs, the decrease in J\textsubscript{H\textsuperscript{+}} ranged from 0.03 to 0.69 mM/pH unit (mean = 0.31 \(\pm\) 0.04; mM/pH unit). Similar results were obtained when the apical membrane was stimulated with HCl solution at pH 3.0 (data not shown). The decrease in J\textsubscript{H\textsuperscript{+}} in the presence of apical acetic acid represents the decrease in H\textsuperscript{+} flux due to the inhibition of the basolateral NHE-1. Taken together, the results suggest that the NHE-1 activity is inhibited by pH\textsubscript{o}. The results further indicate that taste buds are comprised of a heterogeneous population of TRCs that demonstrate variable responses to acid stimuli. Variable responses to acid stimuli may arise due to the differences in resting pH\textsubscript{i}, \(\beta_1\), and NHE-1 activity in individual TRCs. Overall, the data support the hypothesis that only a subset of TRCs act as acid-sensing cells that participate in sour taste transduction.

The mean change in TRC pH\textsubscript{i} (Fig. 15; a-b) for a given change in pH\textsubscript{Ap} (\(\Delta \text{pH}/\Delta \text{pH}\textsubscript{Ap}\)) was 0.055, a value ten fold less than that observed across the basolateral membrane (Figs. 13 and 14). The results suggest that the apical pore region and the paracellular pathway between TRCs present a significant diffusion barrier to acids (Lyall \textit{et al.} 2001). Lowering pH\textsubscript{Ap} decreases resting TRC pH\textsubscript{i} and inhibits pH\textsubscript{i} recovery rate (Vaughan-Jones and Wu 1990; Grinstein and Rothstein 1986), suggesting that during apical acid stimulation the pH\textsubscript{i} recovery by the basolateral NHE-1 is blunted.
DISCUSSION

NHEs constitute a gene family containing several isoforms (NHE-1, NHE-2, NHE-3, NHE-4 and NHE-5), which possess distinct characteristics and serve specialized functions (Josette and Pouysségur 1995). NHEs mediate electro-neutral exchange of Na\(^+\) for H\(^+\) and, thereby, play a central role in pH regulation and Na\(^+\) homeostasis. Our RT-PCR studies provide the first molecular evidence that both NHE-1 and NHE-3 messages are expressed in TRCs (Fig. 1). Using immunocytochemical methods and confocal microscopy, the NHE-3 and NHE-1 were localized to the apical and basolateral membranes of TRCs, respectively (Figs. 2 and 3). NHE-1 has been shown to be present in the basolateral membranes of most epithelial cells (Josette and Pouysségur 1995). The basal NHE-1 activity serves a housekeeping function in maintaining resting pH, by neutralizing internal H\(^+\) ions generated as by products of metabolism (Ritter et al. 2001). NHE-3, the predominant epithelial isoform, is found in the apical membranes of renal and intestinal epithelial cells (Hoogerwert et al. 1996). In our studies, antibodies against NHE-3 that localized to the apical membranes of kidney proximal tubule cells also labeled the apical membranes of TRCs in the fungiform (Fig. 2B) and circumvallate taste buds (Fig. 3D). However, in a subset of TRCs, the label was also present in the intracellular compartment (Fig. 2C). Our dual labeling studies of NHE-3 and the basolateral marker, Na\(^+\)-K\(^+\)-ATPase, demonstrate that in fungiform taste buds, both transporters are present, but do not show any overlap (Fig. 2C). These results indicate that NHE-3 is not present in the basolateral membranes of TRCs.
Our studies demonstrate the presence of a basolateral Na\(^+\)-dependent acid extrusion mechanism in fungiform TRCs that is functional in the nominal absence of CO\(_2\)/HCO\(_3\)^-. The mechanism exchanges intracellular H\(^+\) ions for Na\(^+\) ions (Figs. 4, 10, and 11). Its activity is regulated by Na\(^+\) concentration in the basolateral compartment. The K\(_m\) for Na\(^+\) ranges between 8 and 15 mM (Figs. 5 and 7) and the acid extrusion mechanism is maximally active around 50 mM basolateral Na\(^+\) concentration. At constant pH\(_o\), it is activated by intracellular acidification, and in the nominal absence of CO\(_2\)/HCO\(_3\)^-, is the major mechanism responsible for pH regulation in TRCs (Figs. 6, 9, and 13-15). During pH regulation, it may also serve as a significant pathway for basolateral Na\(^+\) entry (Fig. 10). The basolateral Na\(^+\)-dependent acid extrusion mechanism is inhibited by a specific NHE-1 blocker, HOE642, and by a non-specific blocker of NHEs, amiloride (Fig. 8). At the physiological pH, the acid extrusion mechanism demonstrates low activity since only small changes in resting pH\(_i\) are observed in the presence of the NHE-1 blockers (Figs. 4A and 6).

The rate of TRC pH\(_i\) recovery following an NH\(_4\)Cl pulse was dependent upon the basolateral Na\(^+\) concentration and achieved its maximal rate at around 50 mM Na\(^+\) concentration (cf. Fig. 7). However, increasing basolateral Na\(^+\) concentration from 50 mM to 100 mM produced a small but significant increase in resting TRC pH\(_i\) (cf. Fig. 5). This suggests that at TRC pH\(_i\) above 7.4 there may be Na\(^+\)-dependent pH regulatory pathways, other than NHE-1, that also participate in maintaining the steady-state pH\(_i\) in TRCs. These pathways may
include: a sodium bicarbonate cotransporter, a Na\textsuperscript{+}-dependent chloride-bicarbonate exchanger, or other members of the NHE-family. In addition, at the physiological pH, there is a continuous generation of intracellular H\textsuperscript{+} due to metabolism (Lyall et al. 1997).

The changes in pH\textsubscript{o} have an opposite effect on the basolateral Na\textsuperscript{+}-dependent acid extrusion mechanism. Its activity was enhanced by an increase in basolateral pH (Figs. 13 and 14) while its activity was attenuated when either basolateral pH (Fig. 13) or apical pH was lowered (Fig. 15). However, the data presented in Fig. 13, underestimate the degree to which a decrease in basolateral pH inhibits the pH\textsubscript{i} recovery rate by NHE-1. This is because at the basolateral pH of 6.8, following the NH\textsubscript{4}Cl pulse, the minimum value of pH\textsubscript{i} is lower (Fig. 13; point n) than at pH 7.4 (Fig. 13; point d), and thus, the actual recovery rate at pH 7.4 is less than it would be if the minimum pH at point d were as low as it is at point n. Therefore, a quantitative comparison between the pH\textsubscript{i} recovery rates at different pH\textsubscript{o} values is not possible. A strictly quantitative comparison of pH\textsubscript{i} recovery rates at different pH\textsubscript{o} values can be made if following the NH\textsubscript{4}Cl pulse, the minimum value of pH\textsubscript{i} achieved is the same at each pH\textsubscript{o} value, i.e. if the points d, i, and n in Fig. 13 attain the same pH\textsubscript{i} value. However, the overall point that decreasing pH\textsubscript{o} suppresses the pH\textsubscript{i} recovery rate mediated through NHE-1 is valid.

In contrast to the basolateral NHE-1, the apical NHE-3 seems to be quiescent under the experimental conditions used in this study. The resting TRC pH\textsubscript{i} and the spontaneous pH\textsubscript{i} recovery rates following intracellular acid loading
were not dependent upon apical Na\(^+\) concentration (Figs. 4-6). At present the conditions under which apical NHE-3 is activated in TRCs have not been worked out. It has been demonstrated that in renal brush border, NHE-3 exists in two oligomeric states: a 9.6 S active form and a 21 S inactive form (Biemesderfer et al. 2001). It is likely that NHE-3 exists in an inactive oligomeric state in the apical and intracellular domains of TRCs. Another possibility is that the NHE regulatory factor (NHERF-1), an adaptor necessary for the function of NHE-3 (Reczek et al. 1997), may be lacking in TRCs. Thus apical Na\(^+\) influx does not occur via the apical NHE-3 (Fig. 10). It is well established that Na\(^+\) enters TRCs across the apical membrane via amiloride-sensitive ENaCs (Herness and Gilbertson 1999; Lindemann 2001; Lyall et al. 2002b; Stewart et al. 1997) and by an amiloride-insensitive CPC-sensitive conductive pathway (DeSimone et al. 2001b).

**Implication for sour taste transduction**

The spontaneous pH\(_i\) recovery from an intracellular acid load via the basolateral NHE-1 is activated by a decrease in TRC pH\(_i\) (Figs. 6 and 9) and is inhibited by a decrease in pH\(_o\) (pH\(_{Bl}\) or pH\(_{Ap}\)) (Figs. 13-15). Similar dependence of NHE activity on pH\(_o\) has been reported in other cells (Vaughan-Jones and Wu 1990). The fact that apical stimulation with acids decreases TRC pH\(_i\) has important consequences for sour taste transduction. We have previously shown that acids enter TRCs across the apical cell membranes as neutral molecules (acetic acid, citric acid, and CO\(_2\)) or as H\(^+\) ions (HCl) and produce a sustained decrease in TRC pH\(_i\) (Lyall et al. 2001, 2002a, 2002b). It is hypothesized that
TRCs that respond with maximal decrease in pH$_i$ during acid stimulation participate most in acid transduction. A subset of TRCs may respond with a maximal change in pH$_i$ if the cells possess a low $\beta_1$ and a relatively more alkaline resting pH$_i$. Our data show that within a taste bud there are significant variations between individual TRCs with respect to their resting pH$_i$ and $\beta_1$. Indeed resting pH$_i$ is distributed bimodally among the cells investigated. In one group of cells pH$_i$ is normally distributed about a mean of about 7.2. A second group shows a skewed distribution with a peak at a pH$_i$ of 7.45. The latter group would display the larger sensitivity to acid stimuli. Whether these are the actual transducer cells in sour taste, however, remains to be shown.

During apical acid stimulation sustained decreases in TRC pH$_i$ are observed (Fig. 15) (Lyall et al. 2001, 2002a, 2002b). Sustained changes in pH$_i$ seem to be a common feature of chemosensory cells dedicated to CO$_2$/H$^+$ sensing (Buckler et al. 1991b; Ritucci et al. 1998; Wiemann et al. 1999). The sustained changes in pH$_i$ in chemosensitive neurons (Ritucci et al. 1998; Wiemann et al. 1999) are related to the acid-induced inhibition of pH recovery mechanisms. In ventrolateral medullary neurons and in neurons from the nucleus of the solitary tract (Ritucci et al. 1998) sustained changes in pH$_i$ occur because of the inhibition of NHE. Most of the neurons from the nucleus of the solitary tract and ventrolateral medulla (Ritucci et al. 1997) did not exhibit pH$_i$ recovery when CO$_2$ was increased from 5 to 10% at constant extracellular HCO$_3^-$ concentration (pH$_o$ decreased by 0.3 pH unit; hypercapnic acidosis). However, when CO$_2$ was increased from 5 to 10% at constant pH$_o$ (isohydric hypercapnia), pH$_i$ recovery
was seen. This suggests that the NHE activity in the nucleus of the solitary tract and ventrolateral medullary neurons is inhibited by a decrease in pH$_o$ (Ritucci et al. 1997; Ritucci et al. 1998). It is further suggested that a decrease in the apparent internal H$^+$ ion affinity (pK$_i$) at low pH$_o$ inhibits NHE activity (Vaughan-Jones and Wu 1990). While this may be the mechanism showing how a decrease in pH$_{Bl}$ inhibits basolateral NHE-1 activity in TRCs, the inhibition of basolateral NHE-1 activity from a decrease in pH$_{Ap}$ presumably occurs via additional secondary changes in intracellular cAMP and Ca$^{2+}$ concentration (Lyall et al. 2002a) or via changes in cell volume (Ritter et al. 2001). For example, acid stimulation can cause changes in intracellular Ca$^{2+}$ ([Ca$^{2+}]_i$) concentration (Liu and Simon 2001; Richter et al. 2003). Upon acid stimulation, about 9% of TRCs (Type 1) responded by an increase in [Ca$^{2+}]_i$ and 39% TRCs (Type II) responded with a decrease in [Ca$^{2+}]_i$ (Liu and Simon 2001). A decrease in [Ca$^{2+}]_i$ will most likely result in the inhibition of NHE-1 in a subset of TRCs (Lyall et al. 2002a).

While acid-induced inhibition of pH recovery mechanisms plays an important role in acid-sensing, the activation of pH recovery mechanisms by second messengers plays a role in sour taste adaptation. We have recently demonstrated that an increase in TRC [Ca$^{2+}]_i$ alkalinizes resting TRC pH$_i$ by stimulating NHE activity, which demonstrably increases sensory adaptation to acids (Lyall et al. 2002a). Since changes in [Ca$^{2+}]_i$ modulate the activity of NHE-1 (Josette and Pouysségur 1995; Ritter et al. 2001), it is likely that the isoform NHE-1 is also involved in sour taste adaptation.

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This work was supported by the National Institute of Deafness and other Communications Disorders Grants DC-02422 and DC-00122 (JAD), Department of Veterans Affairs (GMF), VA Career Development Award (AKV) and A.D. Williams grant (AKV). We thank Dr. Nilay V. Desai, Mahdis Mansouri, Leila Z. Islam, and Farrukh M. Hussain for help with imaging studies. We thank Ms. Victoria A. Bickel and Mr. Phillip R. Gunst for help with immunocytochemistry. We thank Dr. Richard M. Costanzo for the use of the Nikon upright fluorescence microscope and digital camera. We thank Dr. Susan Hendricks, Dr. David Hill and Dr. Ammasi Periasamy for help with confocal microscopy at the W.M. Keck Center for Cellular Imaging, University of Virginia, Charlottesville, VA.
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**Figure Legends**

**Fig. 1. RT-PCR for mRNA transcripts encoding NHE-1 and NHE-3 in fungiform taste buds.** Lanes 2-5 show RT-PCR in fungiform taste buds for NHE-1 and lanes (7-10) for NHE-3. The kidney positive controls are shown in lanes 4 and 9. The negative controls without the template are shown in lanes 5 and 10.

**Fig. 2. NHE-3 localization.** NHE-3 antibody binding to the apical membranes of fungiform (FF) TRCs and kidney (K) proximal tubules. Sections were incubated with primary antibodies against rat NHE-3 and Na\(^+\)-K\(^+\)-ATPase. Confocal microscopy revealed that NHE-3 antibody specifically localized on the apical membrane of proximal kidney tubules (A, red label). In FF taste buds, NHE-3 was localized in the apical region (B, red label, arrow). The Na\(^+\)-K\(^+\)-ATPase antibodies (green label) labeled the basolateral membranes of both FF TRCs and proximal tubule cells. (B) Subcellular localization of NHE-3 antibody binding in fungiform (FF) TRCs. Sections were incubated with primary antibodies against rat NHE-3 and Na\(^+\)-K\(^+\)-ATPase. Confocal microscopy revealed that NHE-3 antibody binding is also observed in the intracellular compartment (C, red label) in a sub-set of TRCs. The NHE-3 antibody binding did not co-localize with the Na\(^+\)-K\(^+\)-ATPase (C, green label), indicating that NHE-3 activity is not present in the basolateral cell membranes of TRCs.
Fig. 3. NHE-1 and NHE-3 localization. NHE-1 antibody binding to the basolateral membranes of fungiform (FF) TRCs and kidney (K) collecting duct cells. Confocal microscopy revealed that in FF taste buds, NHE-1 was localized on the basolateral membranes (A, green label) in most TRCs within the taste bud. The NHE-1 antibody specifically localized on the basolateral membrane of kidney collecting duct cells (B, green label). NHE-1 and NHE-3 antibody binding in circumvallate (CV) papilla. Confocal microscopy revealed that NHE-1 antibody was specifically localized on the basolateral membranes of most of the TRCs in the CV papillae (C; green label). In contrast, NHE-3 antibody was localized to the apical region (arrows) of the CV TRCs (D, red label).

Fig. 4. Effect of Na\(^{+}\) removal on TRC pH\(_i\). (A) A lingual epithelial preparation was perfused on both sides with control solution containing 150 mM NaCl (pH 7.4). At the time periods shown by the top horizontal bar the apical ([Na\(^{+}\)]\(_{Ap}\); a-b) or basolateral ([Na\(^{+}\)]\(_{Bl}\); b-c) membrane solution was switched to a Na\(^{+}\)-free solution containing 150 mM NMDG-Cl. In the second step the [Na\(^{+}\)]\(_{Bl}\) was decreased from 150 mM to 0 in the presence of 1 \(\mu\)M HOE642, a specific blocker of NHE-1 (e-f). (B) A lingual epithelial preparation was perfused on both sides with control solution containing 150 mM NaCl (pH 7.4). At the time periods shown by the top horizontal bar the basolateral ([Na\(^{+}\)]\(_{Bl}\); j-k) membrane solution was switched to a Na\(^{+}\)-free solution containing 150 mM NMDG-Cl. In the second step, the [Na\(^{+}\)]\(_{Bl}\) was decreased from 150 mM to 0 in the presence of 10 \(\mu\)M
HOE642, a specific blocker of NHE-1 (m-n). The pH\textsubscript{i} values are presented as mean ± SEM of n (number of ROIs within the taste bud).

**Fig. 5. Effect of step changes in basolateral Na\textsuperscript{+} concentration on TRC pH\textsubscript{i}**. (A) A lingual epithelial preparation was initially perfused on both sides with a solution containing 150 mM NMDG-Cl (pH 7.4). TRC pH\textsubscript{i} was monitored during step changes in basolateral NaCl concentration ([Na\textsuperscript{+}]\textsubscript{Bl}) from 0 to 1.0, 2.5, 5.0, 10, 20, 50, 100, and 150 mM NaCl (equivalent amount of NaCl replaced NMDG-Cl in the basolateral solutions). (B) Relationship between basolateral Na\textsuperscript{+} concentration ([Na\textsuperscript{+}]\textsubscript{Bl}) and TRC pH\textsubscript{i}. The steady-state pH\textsubscript{i} values at each successive Na\textsuperscript{+} concentration from Fig. 5A are plotted in Fig. 5B. The curve was drawn according to a kinetic model of NHE activity (see Appendix). The pH\textsubscript{i} values are presented as the mean ± SEM of n (number of ROIs within the taste bud).

**Fig. 6. Effect of basolateral Na\textsuperscript{+} concentration ([Na\textsuperscript{+}]\textsubscript{Bl}) and HOE642 on the spontaneous TRC pH\textsubscript{i} recovery following acid loading with NH\textsubscript{4}Cl.** A lingual epithelial preparation was initially perfused on both sides with a solution containing 150 mM NaCl (pH 7.4). Temporal changes in TRC pH\textsubscript{i} were monitored following a short basolateral NH\textsubscript{4}Cl pulse (equivalent amount of NaCl replaced NH\textsubscript{4}Cl in the basolateral solutions) under the following conditions: (i) During perfusion of a Na\textsuperscript{+}-free solution (0 Na\textsuperscript{+}) in the basolateral compartment (c-d-e); (ii) During perfusion of control solution containing 1 µM HOE642 (HOE642) in the
basolateral compartment (g-h); and (iii) During perfusion of control solution in the basolateral compartment without HOE642 (j-k). The pH$_i$ values are presented as mean ± SEM of n (number of ROIs within the taste bud).

**Fig. 7.** Relationship between basolateral Na$^+$ concentration ([Na$^+$]$_{Bl}$) and the spontaneous TRC pH$_i$ recovery rate following acid loading with NH$_4$Cl. Temporal changes in TRC pH$_i$ were monitored following a short basolateral NH$_4$Cl pulse in the presence of increasing [Na$^+$]$_{Bl}$ concentrations (equivalent amount of NMDG-Cl replaced NaCl + NH$_4$Cl in the basolateral solutions). In each case the spontaneous recovery of TRC pH$_i$ was monitored following NH$_4$Cl washout ($\delta$pH$_i$/min) and was plotted as a function of [Na$^+$]$_{Bl}$. Increasing the Na$^+$ concentration from 0 to 150 mM, caused the spontaneous pH$_i$ recovery rate to increase as a saturating function of [Na$^+$]$_{Bl}$ ($K_m = 8.3$ mM). This is consistent with the predicted kinetics of NHE (see Appendix).

**Fig. 8.** Effect of NHE-1 blockers on the spontaneous TRC pH$_i$ recovery following acid loading with NH$_4$Cl. The lingual epithelial preparations were initially perfused on both sides with control solution containing 150 mM NaCl (pH 7.4). Temporal changes in TRC pH$_i$ were monitored following exposure of the basolateral membranes to short NH$_4$Cl pulses in the presence of increasing concentrations of amiloride (filled circles), HOE642 (open circles), EIPA (filled squares), and MIA (filled triangles). In each case the rate of spontaneous pH$_i$ recovery in the absence of drug was taken as 100%. The spontaneous pH$_i$
recovery rates are presented as mean ± SEM of N, where N (number of lingual epithelial preparations) = 3.

**Fig. 9. Effect of HOE642 on the spontaneous TRC pH\textsubscript{i} recovery following acid loading with Na-acetate (NaA).** A lingual epithelial preparation was initially perfused on both sides with control solution containing 150 mM NaCl (pH 7.4). Temporal changes in TRC pH\textsubscript{i} were monitored following exposure of the basolateral membranes to short NaA pulses under control conditions (a-b-c) and in the presence of 10 µM HOE642 (e-f-g). The pH\textsubscript{i} values are presented as mean ± SEM of n (number of ROIs within the taste bud).

**Fig. 10. Effect of external Na\textsuperscript{+} concentration on the relative changes in TRC [Na\textsuperscript{+}]\textsubscript{i}.** (A) The TRCs in a lingual epithelium preparation were loaded with Na-green. The tissue was perfused on both sides with control solution containing 150 mM NaCl (pH 7.4). At the time periods shown by the top horizontal bar the apical ([Na\textsuperscript{+}]\textsubscript{Ap}; a-b) or basolateral ([Na\textsuperscript{+}]\textsubscript{Bl}; b-c) membrane solution was switched to a Na\textsuperscript{+}-free solution containing 150 mM NMDG-Cl. The temporal changes in TRC [Na\textsuperscript{+}]\textsubscript{i} were expressed as % mean changes in F\textsubscript{490} ± SEM of n (number of ROIs within the taste bud). (B) The TRCs in a lingual epithelium preparation were loaded with SBFI. The tissue was perfused on both sides with control solution containing 150 mM NaCl (pH 7.4). At the time period shown by the top horizontal bar the basolateral ([Na\textsuperscript{+}]\textsubscript{Bl}; a-b) membrane solution was switched to a Na\textsuperscript{+}-free solution containing 150 mM NMDG-Cl. The temporal changes in TRC [Na\textsuperscript{+}]\textsubscript{i} were
expressed as mean changes in FIR \((F_{340}/F_{380})\) \pm SEM of \(n\) (number of ROIs within the taste bud).

**Fig. 11. Effect amiloride and HOE642 on the relative changes in TRC [Na\(^+\)].**
The TRCs in a lingual epithelial preparation were loaded with Na-green. The tissue was perfused on both sides with control solution containing 150 mM NaCl (pH 7.4). (A) At the time periods shown by the top horizontal bar the basolateral membrane solution was switched to a Na\(^+\)-free solution containing 150 mM NMDG-Cl in the absence (a-b-c) and the presence of 250 \(\mu\)M amiloride (d-e-f) in the basolateral compartment. (B) At the time periods shown by the top horizontal bar the basolateral membrane solution was switched to a Na\(^+\)-free solution containing 150 mM NMDG-Cl in the absence (a-b-c; and g-h-i) and the presence of 10 \(\mu\)M HOE642 (d-e-f) in the basolateral compartment. The temporal changes in TRC [Na\(^+\)] were expressed as % mean changes in \(F_{490}\) \pm SEM of \(n\) (number of ROIs within the taste bud).

**Fig. 12. Measurement of the intrinsic buffering capacity \((\beta_1)\) in TRCs.** (A) A lingual epithelial preparation was initially perfused on both sides with a Na\(^+\)-and Cl\(^-\)-free solution (RONa0Cl; pH 7.4; Table 2) containing 10 mM TEAA. The apical solution, in addition, contained, 2 mM CPC. Temporal changes in TRC pH\(_i\) were monitored following exposure of the basolateral membrane to 30, 20, 10, and 5 mM \((NH_4)_2SO_4\) (equivalent amount of \(K_2SO_4\) replaced \((NH_4)_2SO_4\) in the basolateral solutions). The calculated value of the \(\beta_1\) for step changes in the
basolateral (NH₄)₂SO₄ concentration from 30 to 20, 20 to 10, and 10 to 5 mM is
given above the pHᵢ trace. The pHᵢ values are presented as the mean ± SEM of n
(number of ROIs within the taste bud). The insert shows the relation between
TRC pHᵢ and β₁ in 3 individual taste buds containing 30 ROIs (N = 3; n = 30). The
line of best fit was represented by the equation: β₁ = -36.9 x pHᵢ + 288.6 (r² =
0.92). (B) Resting TRC pHᵢ in different ROIs in the taste buds. The data are
plotted from 7 individual polarized TRC preparations containing 78 ROIs. The
histogram shows the number of ROIs that fall within a given pHᵢ interval.

**Fig. 13. Effect of basolateral pH (pHᵦᵢ) on TRC pHᵢ and the spontaneous pHᵢ recovery rates.** A lingual epithelial preparation was initially perfused on both
sides with control solution containing 150 mM NaCl (pH 7.4). Temporal changes
in TRC pHᵢ were monitored following a short basolateral NH₄Cl pulse (equivalent
amount of NaCl replaced NH₄Cl in the basolateral solutions) when the pHᵦᵢ was
adjusted to 7.4 (a-b-c-d-e), 7.8 (f-g-h-i-j), and 6.8 (k-l-m-n-o). The basolateral
NH₄Cl pulses were applied during the time period shown by the top short
horizontal bars. The pHᵢ values are presented as mean ± SEM of n (number of
ROIs within the taste bud).

**Fig. 14. Relationship between basolateral pH (pHᵦᵢ), TRC pHᵢ, and the spontaneous pHᵢ recovery rates.** The mean ± SEM values of TRC pHᵢ and
δpHᵢ/min from 4 such experiments shown in Fig. 13 are plotted in Fig. 14 against
pHᵦᵢ. The line with slope 0.85 is the predicted relation between pHᵢ and pHₒ.
(filled circles, solid line) based on NHE kinetics (see Appendix). NHE kinetics also predicted that the spontaneous rate of $\text{pH}_i$ recovery is an increasing nonlinear function of $\text{pH}_{\text{Bl}}$. The curve is a least squares fit of the data according to the model developed in the Appendix (open circles, short dashed line).

**Fig. 15. Effect of apical pH ($\text{pH}_{\text{Ap}}$) on TRC $\text{pH}_i$ and the spontaneous $\text{pH}_i$ recovery rates.** A lingual epithelial preparation was initially perfused on both sides with control solution containing 150 mM NaCl (pH 7.4). Temporal changes in TRC $\text{pH}_i$ were monitored following a short basolateral NH$_4$Cl pulse (equivalent amount of NaCl replaced NH$_4$Cl in the basolateral solutions) when the $\text{pH}_{\text{Ap}}$ was adjusted to 7.4 (f-g-h-i) and to 3.0 with acetic acid (b-c-d-e). The basolateral NH$_4$Cl pulses were applied during the time period shown by the short top horizontal bars. The $\text{pH}_i$ values are presented as mean $\pm$ SEM of n (number of ROIs within the taste bud).
APPENDIX

The Na\textsuperscript{+}-H\textsuperscript{+} exchanger, NHE-1, is assumed to be described by the following kinetic scheme (Grinstein and Rothstein 1986).

\[
H_i + Na_o + E \xrightleftharpoons{k_i} HENa \xrightleftharpoons{k_o} H_o + Na_i + E \quad (1)
\]

\(E\) is the membrane-bound exchanger molecule, \(H_i\) and \(Na_i\) and \(H_o\) and \(Na_o\) refer to hydrogen ion and sodium ion inside and outside the cell (basolateral side) respectively, and \(k_i\) and \(k_o\) are rate constants mediating respectively hydrogen ion efflux and influx, through NHE. The complex \(HENa\) is assumed to dissociate in either direction with the same rate constant, \(k_e\). In addition to the \(H^+\) and \(Na^+\) transport sites on the exchanger, there are both internal and external \(H^+\) binding sites that modify the function of the exchanger (Grinstein and Rothstein 1986; Vaughn-Jones and Wu 1990). We assume these modulation sites influence the values of \(k_i\) and \(k_o\) depending on the internal and external \(H^+\) concentration respectively according to:

\[
k_i = k [H_i]^{n-1} \quad (2)
\]

and

\[
k_o = k [H_o]^{m-1} \quad (3)
\]

where \(k, m,\) and \(n\) are constants greater than zero. Using standard methods we obtain:
\[
\frac{dpH_i}{dt} = \frac{r \left( \left[ H_r \right]^n \left[ Na_o \right] - \left[ H_o \right]^n \left[ Na_i \right] \right)}{1 + K \left( \left[ H_r \right]^n \left[ Na_o \right] - \left[ H_o \right]^n \left[ Na_i \right] \right)}
\]  

(4)

where \( r = kE_T/4.606[H_i] \), and \( E_T \) is the total exchanger concentration, and \( K = k/2k_e \).

Introducing pH in Eq. 4 and re-arranging terms gives:

\[
\frac{dpH_i}{dt} = \frac{\rho \left( \left[ Na_o \right] - \left[ Na_i \right] 10^{x} \right)}{\kappa + \left[ Na_o \right] + \left[ Na_i \right] 10^{x}}
\]  

(5)

Here \( x = n \ pHi - m \ pHo \), \( \rho = k_eE_T \ 10^{pHi/2.303} \), and \( \kappa = 2k_e10^{npHi/k} \). When steady state conditions are reached (\( dpH/dt = 0 \))

\[
\ pHi = \frac{m}{n} \ pHo + \frac{1}{n} \log \left( \frac{[Na_o]}{[Na_i]} \right)
\]  

(6)

Equation 6 indicates that when NHE-1 is operating at steady state a linear relation between \( pHi \) and \( pHo \) exists with slope \( (m/n) \). Taking \([Na_o] = 150 \text{ mM} \) and \([Na_i] = 10 \text{ mM} \), a two-parameter least squares fit of the data in Fig. 14 gives \( m = 0.95 \) and \( n = 1.12 \), i.e. a slope of 0.85. The modulation of \( k_i \) and \( k_o \) by pH has the effect, therefore, of attenuating the range of \( pHi \) for a given change in the range of \( pHo \). The same values of these parameters were used with Eq. 5 to fit the initial rate of change of \( pHi \) as a function of \( pHo \). A value of \( k_e/k \) of \( 10^{-7} \) was used because this value also satisfies the fit of the initial rate of change of \( pHi \) as a function of \([Na_o] \) (Fig. 7). A two-parameter fit of the \( dpHi/dt \) data as a function of \( pHo \) is shown in Fig. 14. The parameters were \( \rho = 0.075 \) and \( n \ pHi = 7.48 \). Since \( n = 1.12 \), \( pHi = 6.67 \), which corresponds reasonably well with the value of \( pHi \) observed following an NH\textsubscript{4}\textsubscript{Cl} pulse at the start of the \( pHi \) recovery phase.
Equation 5 was also used to fit $dpH/ dt$ data as a function of $[Na_o]$ at $pH_o = 7.40$ (Fig. 7). The parameter values were $\rho = 0.051$, $\kappa + [Na]10^x = 8.29$ and $[Na]10^x = 2.15$. If we assume $n = 1.12$, and $pH_i = 6.67$, as in the fit of $dpH/ dt$ as a function of $pH_o$, then $\kappa = 6.14$, from which it follows that $k_e/k$ of $10^{-7}$ as before.

Equation 6 was also used to fit the steady state $pH_i$ as a function of $[Na_o]$ at $pH_o = 7.4$ using the same parameters for $m$, $n$, and $[Na]$ used to fit the data in Figs. 7 and 14. However, above a $[Na_o]$ of 20 mM the model consistently over-estimated the observed steady state values of $pH_i$. This result suggests that at a given value of $[Na_o]$ as the steady state is approached both $m$ and $n$ increase slightly with pH thereby preventing the cell from becoming excessively alkaline. This can be illustrated by assuming:

$$n = \alpha pHi \quad (7)$$

and

$$m = \beta pH_o \quad (8)$$

where $\alpha$ and $\beta$ are pH-independent constants and substituting into Eq. 6, viz.:

$$pHi = \sqrt{(\beta/\alpha)(pH_o)^2 + (1/\alpha)\log([Na_o]/[Na_i])} \quad (9)$$

To fit the steady state data in Fig. 5, $pH_o = 7.4$ and we used $[Na] = 10$ mM as before. The least-squares values of $\alpha$ and $\beta$ were 0.15 and 0.14 respectively.

The dependence of TRC $pH_i$ as a function of $pH_o$ and $[Na_o]$ is, therefore, consistent with the kinetic predictions of a cell membrane sodium-hydrogen exchanger.
### TABLE 1

**Solution Composition**

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R = Ringer’s solution; RC = control solution; R0Na = 0 Na⁺ solution; RNH₄Cl = 30 mM NH₄Cl solution; RNaA = 30 mM sodium acetate solution; RAA = 58.3 mM acetic acid solution.

NaPy = sodium pyruvate; NaA = sodium acetate; NMDGCl = N-methyl-D-glucamine chloride; HEPES = N-(2-hydroxyethyl)piperazine-N’-(2-ethanesulfonic acid); AA = acetic acid.

*In some solutions 1 mM HCl was used instead of AA (RHCl; pH 3.0).
TABLE 2

Solution Composition

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R0Na0Cl = Na⁺- and Cl⁻-free solution; R(NH₄)₂SO₄ = Na⁺- and Cl⁻-free solution containing 20 mM (NH₄)₂SO₄; CS = intracellular pH calibrating solutions adjusted to pHs between 6.5 and 8.0, HEPES = N-(2-hydroxyethyl)piperazine-N’-(2-ethanesulfonic acid); TEAA = tetraethylammonium acetate.
*The apical solution, contained, in addition, 2 mM cetylpyridinium chloride (CPC).
Fig. 3
Fig. 4
Fig. 5
Fig. 6
Fig. 7
Fig. 10
Fig. 11
Fig. 12
Fig. 13