The K^+-H^+ Exchanger, Nigericin, Modulates Taste Cell pH and Chorda Tympani Taste Nerve Responses to Acidic Stimuli

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Abstract

The relationship between acidic pH, taste cell pH_i, and chorda tympani (CT) nerve responses was investigated before and after incorporating the K^+-H^+ exchanger, nigericin, in the apical membrane of taste cells. CT responses were recorded in anesthetized rats in vivo, and changes in pH_i were monitored in polarized fungiform taste cells in vitro. Under control conditions, stimulating the tongue with 0.15 M potassium phosphate (KP) or 0.15 M sodium phosphate (NaP) buffers of pHs between 8.0 and 4.6, KP or NaP buffers did not elicit a CT response. Post-nigericin (500×10^{-6} M), KP buffers, but not NaP buffers, induced CT responses at pHs ≤ 6.6. The effect of nigericin was reversed by the topical lingual application of carbonyl cyanide 3-chloro-phenylhydrazone, a protonophore. Post-nigericin (150×10^{-6} M), KP buffers induced a greater decrease in taste cell pH_i relative to NaP buffers and to NaP and KP buffers under control conditions. A decrease in pH_i to about 6.9 induced by KP buffers was sufficient to elicit a CT response. The results suggest that facilitating apical H^+ entry via nigericin decreases taste cell pH_i and demonstrates directly a strong correlation between pH_i and the magnitude of the CT response.

Key words: CCCP, pH threshold, pH_i, pH_o, sour taste

Introduction

Sour taste is uniquely elicited by acidic stimuli. Both strong and weak organic acids taste sour to humans. Sour taste is generally aversive and helps us to avoid spoiled foods or unripened fruit. Along with the lungs and the kidneys, which are the primary organs for acid secretion, sour taste may also play an important role in maintaining acid-base homeostasis by preventing the ad libitum ingestion of acid (Roper 2007). In the taste bud, type III cells (or presynaptic cells) that express the polycystic kidney disease-like proteins PKD2L1 and/or PKD1L3, function as sour-sensing cells (Huang et al. 2006; Ishimaru et al. 2006; LopezJimenez et al. 2006; Roper 2007; Chandrashekar et al. 2009; Huque et al. 2009; Ishimaru and Matsunami 2009). PKD2L1 (Horio et al. 2010) but not the PKD1L3 protein is essential for normal sour taste transduction in the anterior tongue innervated by the chorda tympani (CT) taste nerve (Yoshida et al. 2009; Horio et al. 2010; Nelson et al. 2010). In polarized fungiform taste bud preparations, stimulating the apical membrane with strong or weak organic acids decreased in-tracellular pH (pH_i) (Stewart et al. 1998; Lyall et al. 2001, 2004, 2006; Lyall, Alam, Phan, Phan, et al. 2002; Lyall, Alam, Phan, Russell, et al. 2002). Only a small subset of taste bud cells specifically responded to this ΔpH_i with a sour transduction signal, a Ca^{2+} transient generated by Ca^{2+} influx through voltage-gated calcium channels consequent to membrane depolarization (Richter et al. 2003). Both the CT response to CO_2 (a weak organic acid) buffered to pH 7.4 with $KHCO_3$ (Lyall et al. 2001; Chandrashekar et al. 2009) and the CO_2-induced decrease in taste cell pH_i are attenuated in the presence of a membrane permeable blocker of intracellular carbonic anhydrase (Lyall et al. 2001). CT responses to CH_3COOH are independent of stimulus pH but strongly correlate with the decrease in pH_i of polarized fungiform taste cells (Lyall et al. 2001). These results suggest that an acid-induced decrease in taste cell pH_i is an important first step in sour taste transduction. However, at present, the relationship between stimulus pH (pH_o), changes in taste cell pH_i, and the magnitude of the

CT response has not been examined in detail over the physiological range of pH for acidic stimuli commonly encountered in food and beverages.

In this paper, the relationship between pH_o, taste cell pH_i, and the magnitude of the CT response was investigated after incorporating the K^+-H^+ exchanger nigericin in the apical membrane of rat fungiform taste bud cells. CT responses were monitored while the rat tongue was stimulated with 0.15 M potassium phosphate (KP) or sodium phosphate (NaP) buffers adjusted to a pH between 8.0 and 4.6. In parallel experiments, changes in taste cell pH_i induced by KP or NaP buffers were monitored in polarized fungiform taste cells in vitro using 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF-AM). Our results show that, under control conditions, KP or NP buffers and NP buffers post-nigericin treatment did not elicit a CT response between pH 8.0 and 4.6. Following nigericin (500×10^{-6} M) treatment, KP buffers induced distinct phasic and tonic CT responses at pH ≤ 6.6 and shifted the threshold for eliciting a CT response with 0.15 M potassium citrate (KC) buffers from pH 2.6 to pH 3.9. The effect of nigericin on the CT responses was reversed by the topical lingual application of carbonyl cyanide 3-chloro-phenylhydrazone (CCCP), a protonophore (H^+ ionophore). The electrogenic H^+ conductance through CCCP most likely depolarizes membrane potential and inhibits the ΔpH_i-induced increase in Ca^{2+} influx in sour-sensing taste cells through voltage-gated Ca^{2+} channels (Roper 2007). Post-nigericin (150×10^{-6} M) treatment, a decrease in pH_i below about 6.9 induced by the KP buffers was sufficient to elicit a CT response. This suggests that facilitating H^+ entry across taste cell apical membrane via the K^+-H^+ exchanger nigericin decreased taste cell pH_i below the threshold pH for evoking CT responses. The magnitude of the phasic and tonic CT responses varied with the nigericin concentration and the graded decrease in taste cell pH_i induced by KP buffers. These results provide further evidence that an acid-induced decrease in taste cell pH_i is an important first step in sour taste transduction. Some of the data in this paper have been published earlier as an abstract (Lyall et al. 2007).

Materials and methods

In vivo studies

CT taste nerve recordings

The animals were housed in the Virginia Commonwealth University animal facility in accordance with institutional guidelines. All in vivo and in vitro animal protocols were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. Female Sprague–Dawley rats (150–200 gm) were anesthetized by intraperitoneal injection of pentobarbital (0.06 gm/kg), and supplemental pentobarbital (0.02 gm/kg) was administered as necessary to maintain surgical anesthesia. The animal's corneal reflex and toe-pinch reflex were used to monitor the depth of surgical anesthesia. Body temperatures were maintained at 37 °C with a Deltaphase Isothermal PAD (Model 39 DP; Braintree Scientific, Inc.). The left CT nerve was exposed laterally as it exited the tympanic bulla and placed onto a 32G platinum/iridium wire electrode (Lyall et al. 2001, 2004, 2006, 2009; Lyall, Alam, Phan, Phan, et al. 2002; Lyall, Alam, Phan, Russell, et al. 2002).

The composition of various stimulating solutions used in the CT experiments is given in Table 1. The various drugs and their concentrations used and their physiological targets are given in Table 2. The anterior lingual surface was stimulated with 0.15 M KP or NaP buffers adjusted to pH between 8.1 and 4.6 (Table 1) (Gomori 1955). The K^+ or Na^+ concentration in KP or NaP buffers of varying pHs was maintained at 0.15 M by additional KCl or NaCl as needed. The KP or NaP buffered solution at pH 8.1 was used as the rinse solution. Before the start of CT recording, the lingual surface was superfused with rinse solution for 10 min. CT responses were monitored before and after the topical lingual application of nigericin (Table 2) for 30 min. Following nigericin treatment, the lingual surface was again superfused with rinse solution for 10 min. Nigericin, a polyether antibiotic from the fungus *Streptomyces hygroscopicus*, is a carboxylic ionophore that exchanges H^+ for a cation, such as K^+ or Na^+ across cell membranes (Pressman 1968). The ionophore has a lower affinity for Na^+ than for K^+ (Pressman 1976). It equilibrates pH_i and pH_o in the presence of high extracellular K^+ (Supplementary Figure 3). The change in CT response in the absence and presence of KP buffers was compared with changes observed using equivalent NaP buffers. In some experiments, CT responses were recorded while the rat tongue was stimulated with 0.15 M potassium citrate (KC) solutions buffered to pHs between 8.3 and 2.6 by titrating with 0.15 M citric acid (Table 1) before and after nigericin treatment. CT responses were also monitored after topical lingual application of carbonyl cyanide 3-chloro-phenylhydrazone (CCCP, a H^+ ionophore), 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis(acetoxymethyl ester) (BAPTA-AM, an intracellular Ca^{2+} chelator) and ionomycin (a Ca^{2+} ionophore) (Table 2). Typically, stimulus solutions remained on the tongue for 2 min. Control stimuli consisting of 0.3 M NaCl and 0.3 M NH_4Cl applied at the beginning and at the end of the experiment were used to assess preparation stability (Figure 1A,B and Supplementary Figure 2). The preparation was considered stable, only if, the difference between the magnitude of the responses to control stimuli at the beginning and at the end of the experiment was less than 10% (Lyall et al. 2009). In some experiments, CT responses were recorded while the tongue was stimulated with sweet (0.5 M sucrose, 0.25 M glycine or 0.005 M SC45647), bitter (0.01 M quinine), umami (0.1 M monosodium glutamate [MSG] + 5×10^{-6} M benzamil [Bz] + 1×10^{-6} M SB366791 [SB],

Table 1 Solution composition

Solution	Composition (M)	pH
Rinse (R)	0.01 KCl	
Control-1	0.3 NH_4Cl	
Control-2	0.3 NaCl	
Sweet stimuli	Sucrose (0.5), glycine (0.25); SC45647 (0.005)	
Bitter stimuli	Quinine (0.02)	
Umami stimuli[a]	0.1 M MSG + 5×10^{-6} Bz + 1×10^{-6} SB; MSG + Bz + SB + 0.001 IMP	
Sour stimuli	H_3PO_4 (0.0005–0.02), HCl (0.0005–0.02), CH_3COOH (0.0005–0.03)	
KP buffers[b]	0.15 K_2HPO_4/0.15 KH_2PO_4 + KCl	8.1–4.5
NaP buffers[b]	0.15 Na_2HPO_4/0.15 NaH_2PO_4 + NaCl	8.1–4.5
KC buffers[b]	0.15 M potassium citrate/0.15 M citric acid + KCl	8.3–2.6
Ringer's solution	0.14 NaCl + 0.005 KCl + 0.001 $CaCl_2$ + 0.001 $MgCl_2$ + 0.01 Na-pyruvate + 0.01 glucose + 0.01 HEPES	7.4
HK calibrating solution	0.0046 NaCl + 0.14 KCl + 0.002 $CaCl_2$ + 0.001 $MgCl_2$ + 0.01 glucose + 0.01 HEPES	6.6–7.8

[a]Benzamil (Bz) is a specific blocker of ENaC and SB-366791 (SB) blocks TRPV1t (Lyall et al. 2009).
[b]In KP, KC, and NaP buffers of varying pHs, the K^+ and Na^+ concentrations were maintained at 0.15 M by the addition of KCl and NaCl as needed.

Table 2 List of drugs used in the study and their physiological targets

Drug	Concentration (M)	Target	Number of animals
Nigericin (in vivo)	75×10^{-6} to 500×10^{-6}	Acts as K^+-H^+ exchanger in the membrane	42
Nigericin (in vitro)	150×10^{-6}	Acts as K^+-H^+ exchanger in the membrane	4
Carbonyl cyanide 3-chloro-phenylhydrazone (CCCP)	150×10^{-6} to 500×10^{-6}	Protonophore (H^+ ionophore)	12
$ZnCl_2$	0.01 M	Zn^{2+} is a nonspecific blocker of H^+ channels	9
Benzamil (Bz)	5×10^{-6}	Bz is a specific blocker of ENaC	3
SB-366791	1×10^{-6}	SB is a specific blocker of TRPV1t	3
BAPTA-AM	0.1×10^{-3} to 33×10^{-3}	Intracellular Ca^{2+} chelator	3
Ionomycin	150×10^{-6}	Ca^{2+} ionophore	3
Inosine 5′ monophosphate (IMP)	1×10^{-3}	IMP specifically enhances CT response to MSG	3

In CT experiments, nigericin, BAPTA-AM, and CCCP were dissolved in 2 mL dimethyl sulfoxide (DMSO) and applied topically to the tongue for 30 min. Under control conditions, CT responses were monitored after topical lingual application of 2 mL DMSO for 30 min. Stock solutions of Bz and SB were made in DMSO and were added to the MSG solutions to achieve a final concentration of 5×10^{-6} and 1×10^{-6} M, respectively. All drugs were purchased from Sigma. For pH-imaging studies, anterior lingual epithelium containing fungiform taste buds was harvested from 15 rats.

and MSG + Bz + SB + 0.001 M inosine 5′-monophosphate [IMP]) and sour stimuli (HCl, H_3PO_4, or CH_3COOH) relative to 0.01 M KCl rinse (Table 1). Benzamil (Bz) is a specific blocker of ENaC and SB-366791 (SB) blocks TRPV1t (Lyall et al. 2009). Bz and SB together block the entire contribution of Na^+ to the CT response to MSG.

The data were digitized and analyzed off line. Both transient (phasic) and tonic (steady-state) parts of the CT responses to KP and NaP buffers were quantified. We also quantified the transient (phasic) response to the application of rinse solution (R) to a tongue already superfused with R.

To quantify the phasic part of the CT response, the height of the stimulus-induced maximum CT response relative to baseline response was divided by the mean steady-state (tonic) response to 0.3 M NH_4Cl. To quantify the tonic (steady-state) part of the CT response, the area under the response versus time curve was taken over the final 30 s of the response. To normalize, this area was divided by the area under the 0.3 M NH_4Cl response curve over the final 30 s of the tonic response period. The normalized data were reported as mean ± standard error of the mean (SEM) of the number of animals. Student's t-test was employed to analyze the
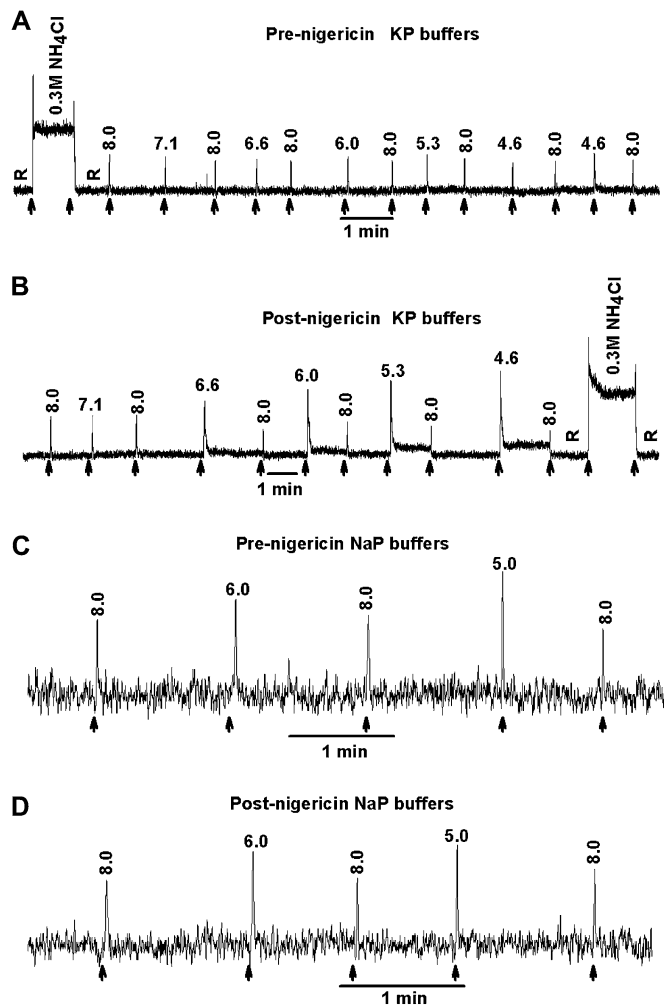
Figure 1 Effect of KP buffers on CT responses before and after topical lingual application of nigericin. **(A, B)** Figure shows a representative CT recording in which the rat tongue was first rinsed with the KP buffer at pH 8.0 and then with KP buffers adjusted to pH 7.1, 6.6, 6.0, 5.3, and 4.6 before (A; pre-nigericin KP buffers) and after topical lingual application of nigericin (500×10^{-6} M) (B; post-nigericin KP buffers). The control responses to 0.3 M NH_4Cl are shown at the beginning and end of the experiment and were nearly identical. The arrows show the time periods when the lingual surface was superfused with different KP buffers. **(C, D)** Figure shows a representative CT recording in which the rat tongue was first rinsed with the NaP buffer at pH 8.0 and then with NaP buffers adjusted to pH 6.0 and 5.0 before (C; pre-nigericin NaP buffers) and after topical lingual application of nigericin (500×10^{-6} M) (D; post-nigericin NaP buffers). In Figure 1C,D, the corresponding control responses to 0.3 M NH_4Cl are not shown, and thus, the scale is different from that in Figure 1A,B.

differences between sets of data. Because we are comparing the normalized CT responses before and after nigericin treatment in the same CT preparation, paired t-test was used to evaluate statistical significance (Lyall et al. 2009).

Following nigericin treatment, the data points on the graphs of the mean normalized phasic and tonic responses of KP buffers as a function of pH_o described a sigmoid similar to that of a titration curve and were accordingly fit to

a mathematical model of that form according to a least squares fit criterion. For tonic responses, the curve was:

$$R = \frac{R_m}{1 + 10^{(pH_o - pH_{o(0.5)})}} \quad (1)$$

Where, R is the normalized tonic CT response, R_m is the maximum response, pH_o is the extracellular pH, and $pH_{o(0.5)}$ is the pH at which R equals $0.5R_m$. For phasic responses, the model was similar to equation (1) except for a nonzero asymptotic value, R_b, at high pH that represents the baseline established by the mechanical artifact upon stimulus addition, that is:

$$R = \frac{R_a}{1 + 10^{(pH_o - pH_{o(0.5)})}} + R_b \quad (2)$$

Here, R_m equals ($R_a + R_b$). In the absence of nigericin tonic, CT responses were fit to $R = apH_o$. Here, the slope a was always at or near zero. For phasic responses in the absence of nigericin, $R = apH_o + R_b$. Similarly, a was always at or near zero, and R_b was the nonzero asymptote.

Following nigericin treatment, for normalized tonic responses of KP buffers as a function of pH_i we used:

$$R = \frac{R_m}{1 + 10^{n(pH_i - pH_{o(0.5)})}} \quad (3)$$

Where, R, R_m, and $pH_{o(0.5)}$ are as previously defined, pH_i is the intracellular pH, and n is a number greater than one that reflects the much smaller allowed range of variation observed in pH_i compared with pH_o. The larger n, the smaller must be the change in pH_i to produce large changes in R. In this respect, large n may be regarded as a measure of the increased sensitivity to small changes in pH that accrues to the sour-sensing taste cells as they function by mapping the large range in stimulus pH_o to the much narrower allowed range of pH_i. For example, in Figure 6E, the post-nigericin data points are described by equation (3) with $R_m = 0.165$, $pH_{o(0.5)} = 6.73$, and $n = 7.0$. For the phasic response dependence on pH_i, equation (3) was modified as in equation (2) to include the nonzero asymptote:

$$R = \frac{R_a}{1 + 10^{n(pH_i - pH_{o(0.5)})}} + R_b \quad (4)$$

In Figure 6D, $R_a = 0.31$, $R_b = 0.76$, $pH_{o(0.5)} = 6.80$, and $n = 8.3$.

Because direct measurements of pH_i could not be made in vivo, the pH_i values used in Figure 6D,E were calculated based on measurements of pH_i as a function of pH_o in vitro in the absence of nigericin and in the presence of 150×10^{-6} M nigericin (see next section). The results in each case (Figure 6C) were fitted to a second order equation of the form:

$$pH_i = c + apH_o + b(pH_o)^2 \quad (5)$$

For the nigericin treated case, $a = -0.667$, $b = 0.712$, and $c = 8.18$. For control conditions, $a = -0.088$, $b = 0.014$, and $c = 7.15$.

In vitro studies

pH_i measurements in polarized fungiform taste cells

Female Sprague–Dawley rats (150–200 gm) were anesthetized by exposing them to the inhalation anesthetic, isoflurane (1.5 mL) in a desiccator. When rats were fully unconscious, a midline incision was made in the chest wall and the aorta severed. The tongues were then rapidly removed and stored in ice-cold normal Ringer's solution (Table 1). The lingual epithelium was isolated by collagenase treatment. A small piece of the anterior lingual epithelium containing a single fungiform papilla was mounted in a special microscopy chamber as described earlier (Lyall et al. 2001, 2004, 2006; Lyall, Alam, Phan, Phan, et al. 2002; Lyall, Alam, Phan, Russell, et al. 2002).

The changes in taste cell pH_i were monitored using the pH-sensitive dye, 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF; Molecular Probes) as temporal changes in the fluorescence intensity ratio (F_{490}/F_{440}) (Lyall et al. 2001, 2006), whereas the apical membrane of the taste cells was perfused with KP or NaP buffered solution adjusted to pHs between 8.0 and 4.6. The basolateral side was continuously perfused with control Ringer's solution (pH 7.4; Table 1). Taste cell pH_i was monitored before and after treating the apical membrane with 150×10^{-6} M nigericin. Similar to the CT experiments, before measuring changes in pH_i, the apical membrane of taste cells was superfused with the KP or NaP buffer of pH 8.0 for 10 min. At the end of each experiment, the changes in taste cell pH_i were calibrated by bilateral perfusion of high K^+ solutions containing 10×10^{-6} M nigericin adjusted to pHs between 8.0 and 6.5 (Table 1).

Results

Effect of nigericin on CT responses to KP and NaP buffers

Under control conditions, stimulating the tongue with KP buffers of pHs 8.0, 7.1, 6.6, 6.0, 5.3, and 4.6 produced only rapid transient upward deflections in the baseline neural record that were pH_o independent (Figure 1A). These transients represent the mechanical rinse artifact of superfusing the tongue with test solutions (Lyall et al. 1999). Similarly, only transient mechanical rinse artifacts were observed with NaP buffers at pH 8.0, 6.0, and 5.0 (Figure 1C). Post-nigericin treatment, a CT response comprising both a phasic and a tonic component could be readily observed by stimulating the tongue with the KP buffer of pH 6.6 (Figure 1B). The magnitudes of both the normalized tonic (Figure 2A; ●) and phasic (Figure 2B; ●) components of the CT responses increased with a further lowering of the KP buffer pH. In contrast, under control conditions, stimulating the tongue with KP or NaP buffers (Figure 2A; ▲ and Figure 2B; ○) or post-nigericin treatment stimulating the tongue with NaP

buffers (Figure 2A; ○ and Figure 2B; ○) did not elicit phasic or tonic CT responses.

The post-nigericin normalized phasic component of the CT response to KP buffers increased with a decrease in pH_o and saturated between pH_o 5.3 and 4.6 (Figure 2B; ●). Post-nigericin treatment (500×10^{-6} M), the mean pH_o at which the normalized phasic CT response was 50% of the maximum response ($pH_{o(0.5)}$) was 6.5. With increasing nigericin concentration, the pH_o versus the normalized tonic CT response relationship for KP buffers was shifted to the right on the pH_o axis relative to control (Figure 2C). The mean $pH_{o(0.5)}$ at 150×10^{-6}, 250×10^{-6}, and 500×10^{-6} M nigericin was 5.57 ± 0.09, 6.05 ± 0.12, and 6.17 ± 0.07, respectively. At 75×10^{-6} M nigericin, $pH_{o(0.5)}$ is difficult to determine accurately, but it is clearly much less than 5. A least squares fit of the data yielded a mean value of 2.71.

CT responses to H_3PO_4, CH_3COOH, and HCl

Distinct phasic and tonic CT responses were observed starting at 0.0005 M H_3PO_4 (Figure 3A). Increasing the H_3PO_4 concentration to 0.001, 0.01, and 0.02 M produced a concentration-dependent increase in the CT response. The pH values of 0.0001, 0.001, 0.01, and 0.02 M H_3PO_4 are 4.01, 3.05, 2.24, and 2.04, respectively. These results suggest that the threshold pH of a rat CT response to H_3PO_4 is around pH 4.0. Similarly, stimulating the tongue with HCl or CH_3COOH produced a concentration-dependent increase in the CT response. In each case, the relationship between the acid's pH value and the tonic CT response resembles that of a titration curve (Figure 3B). Among the 3 acids, CH_3COOH was the strongest sour taste stimulus. This is because most of the CH_3COOH is present in the membrane permeable undissociated form (Lyall et al. 2001). It enters the cell passively and dissociates intracellularly to produce H^+ and CH_3COO^-. Consistent with this, CT responses to CH_3COOH were not affected by the presence of 0.01 M Zn^{2+} (Supplementary Figure 1B), a nonspecific blocker of proton channels (DeCoursey 2010). Zn^{2+} inhibited the HCl CT response to baseline (Supplementary Figure 1B), suggesting that in taste cells, the CT response to HCl depends upon H^+ influx via apical proton channels (Lyall et al. 2004). Zn^{2+} blocked about 66% of the H_3PO_4 CT response (Supplementary Figure 1B), suggesting that H_3PO_4 behaves primarily like a strong acid (i.e., dissociated into H^+ and $H_2PO_4^-$), but unlike HCl, there remains a significant amount of the undissociated form, which like CH_3COOH crosses taste cell apical membranes as the membrane permeable undissociated acid. It would appear, therefore, that protons yielded by HCl and H_3PO_4 permeate the apical taste cell membrane through the same Zn^{2+}-sensitive proton channels.

Following nigericin (500×10^{-6} M) treatment, the magnitude of the normalized tonic CT response to KP buffer of pH 6.18 was equivalent to the normalized tonic CT response obtained with H_3PO_4, HCl, or CH_3COOH with a pH of 2.24, 2.0, or 3.38, respectively (Figure 3C). After nigericin, the magnitude
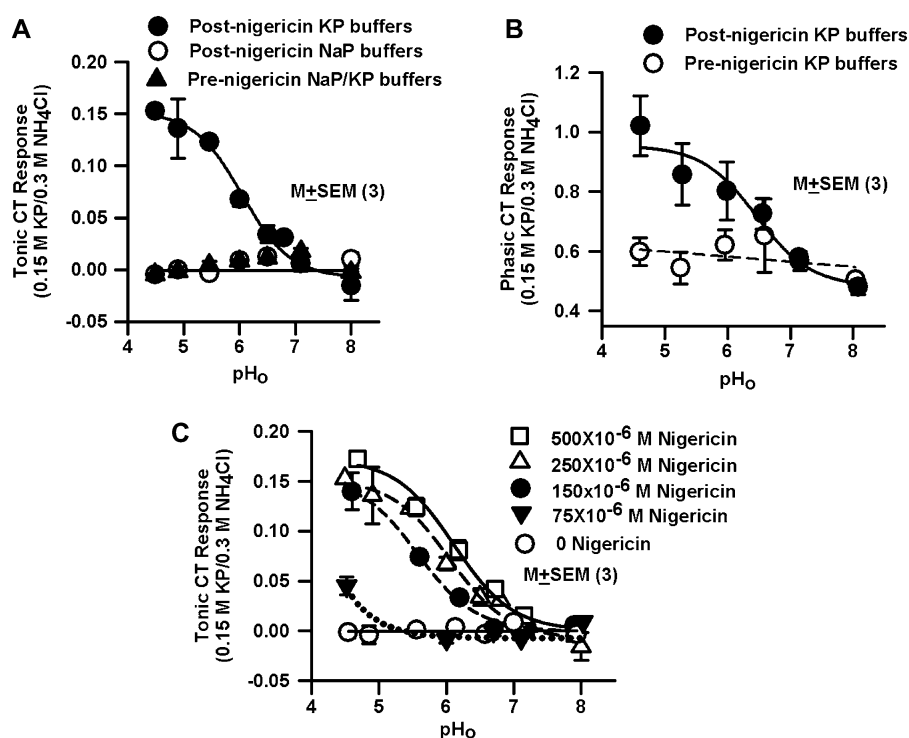
Figure 2 Effect of KP or NaP buffers on the phasic and tonic CT responses before and after topical lingual application of nigericin. **(A)** Summarizes the effects of KP and NaP buffers on the tonic CT responses before and after nigericin (500×10^{-6} M) treatment. **(B)** Summarizes the effects of KP and NaP buffers under control conditions and post-nigericin (500×10^{-6} M) treatment on the phasic component of the CT responses to KP buffers. **(C)** Summarizes the effect of increasing nigericin concentrations (75×10^{-6} to 500×10^{-6} M) on the tonic component of the CT response to KP buffers. Each point represents the normalized mean ± SEM phasic or tonic CT response (0.15 M KP or NaP CT response/0.3 M NH_4Cl) from 3 animals.

of the normalized tonic CT response to KP buffer of pH 4.68 was equivalent to the normalized tonic CT response obtained with H_3PO_4, HCl, or CH_3COOH with a pH of 2.04, 1.70, and 3.0, respectively (Figure 3C). Thus, at high nigericin concentrations, H^+ entry via the K^+-H^+ exchanger induced by KP buffers is comparable to acid entry via proton channels and/or via passive diffusion as the undissociated acid.

We also tested the effect of nigericin on the CT responses to HCl, H_3PO_4, and CH_3COOH solutions in deionized water (i.e., in the absence of external Na^+ or K^+). Post-nigericin (500×10^{-6} M) treatment, the mean normalized tonic CT response to 0.02 M HCl was significantly greater relative to its value under control conditions (Figure 3D). Nigericin did not alter tonic CT responses to 0.02 M H_3PO_4 or 0.02 M acetic acid (Figure 3D).

Effect of nigericin on CT responses to KC and NaC buffers

The CT responses were also recorded while the rat tongue was first superfused with the KC buffer rinse solution (R1; pH 8.2) and then with KC buffers of pHs 5.8, 5.1, 4.5, 3.9, 3.4, and 2.6. Under control conditions, the CT response comprising both phasic and tonic components was observed only at pH 2.6 (Figure 4A). Similar results were obtained with NaC buffers (data not shown). Post-nigericin treatment (500×10^{-6} M), decreasing the pH of the KC buffers, the CT response could be readily observed at pH 3.9

(Figure 4B). The phasic and the tonic components of the CT response increased with a further lowering of pH_o of the KC buffers in a concentration-dependent manner saturating around pH_o 2.6 (Figure 4B). The CT response with NaC buffers post-nigericin treatment was obtained only at pH 2.6 (data not shown). In 3 animals, the normalized phasic response $pH_{o(0.5)}$ shifted from 2.93 in the absence of nigericin to 3.88 in the presence of 500×10^{-6} M nigericin (Figure 4C). The post-nigericin (500×10^{-6} M) pH_o versus tonic CT response curve using KC buffers was shifted to the right by 1.3 pH units. The $pH_{o(0.5)}$ value for the normalized tonic CT response shifted from 2.28 in the absence of nigericin to 3.58 in the presence of 500×10^{-6} M nigericin (Figure 4D). Post-nigericin treatment, with KC buffers, a significantly smaller shift in the threshold pH was observed relative to KP buffers. This effect is most likely due to the binding of citrate ions to $[Ca^{2+}]_i$ and inhibition of the ΔpH_i-induced increase in $[Ca^{2+}]_i$ necessary for the sour taste transduction (see also Figure 7 below) (Richter et al. 2003; Lyall et al. 2006).

Effect of CCCP on the CT response to KP buffers

CCCP has been shown to produce intracellular acidification due to an H^+ conductance induced by the protonophore (Meech and Thomas 1980). Accordingly, CCCP should also enhance the magnitude of the CT response to acidic stimuli and shift the relationship between pH_o and CT response to
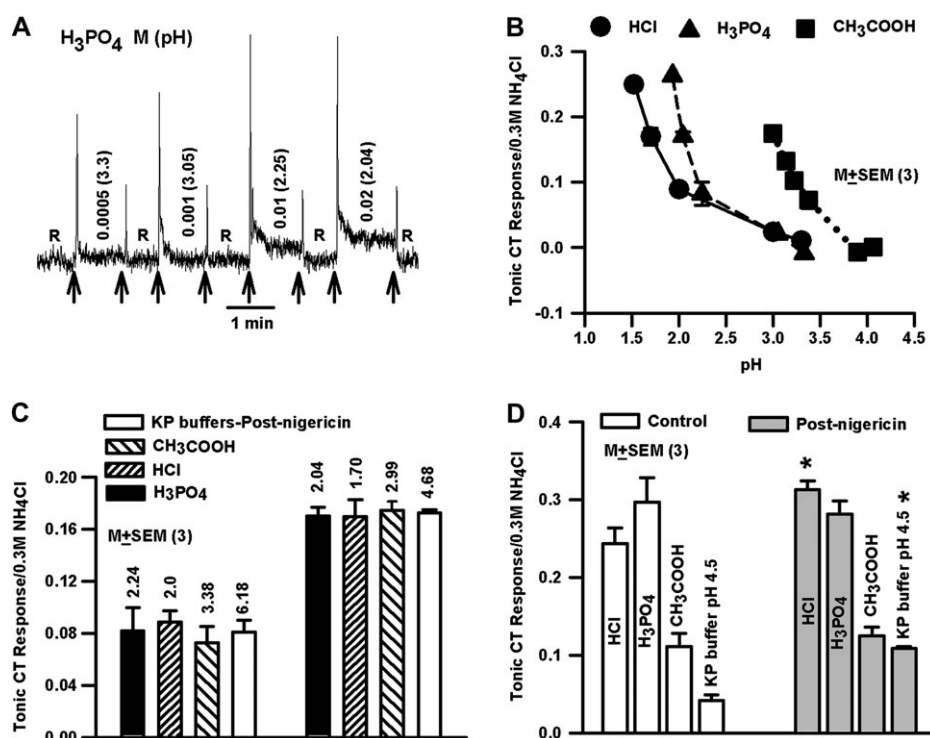
Figure 3 Effect of H_3PO_4, CH_3COOH, and HCl on CT responses. **(A)** Figure shows a representative CT recording in which the rat tongue was first rinsed with the rinse solution (R; 0.01 M KCl) and then with 0.0005, 0.001, 0.01, and 0.02 M H_3PO_4. **(B)** Shows mean normalized tonic CT responses to increasing concentrations of H_3PO_4, CH_3COOH, and HCl plotted as a function of pH. **(C)** Shows the corresponding H_3PO_4, CH_3COOH, and HCl pHs that produce equivalent CT responses to KP buffers post-nigericin (500×10^{-6} M) treatment at pH 6.18 and 4.62, respectively. Each bar represents the normalized mean ± SEM tonic CT response (tonic CT response/0.3 M NH_4Cl) from 3 animals. **(D)** Shows the rat tonic CT responses to 0.02 M HCl, 0.02 M H_3PO_4, and 0.02 M CH_3COOH before (control) and after topical lingual application of 500×10^{-6} M nigericin (post-nigericin). Each bar represents the normalized mean ± SEM tonic CT response (tonic CT response/0.3 M NH_4Cl) from 3 animals.

lower concentrations of acids (i.e., higher pH values) relative to control. In contrast, treating the tongue with 500×10^{-6} M CCCP, post-nigericin (500×10^{-6} M) treatment (Figure 5B), inhibited both the phasic and tonic CT responses to KP buffers at pH 6.5, 6.0, 5.5, and 4.6 relative to post-nigericin treatment alone (Figure 5A). CCCP inhibited the post-nigericin (500×10^{-6} M) phasic (Figure 5C) and tonic (Figure 5D) CT responses to KP buffers in a concentration-dependent manner. There were no differences in the phasic responses in the presence of either 250×10^{-6} (Figure 5C; ▲) or 500×10^{-6} M CCCP (Figure 5C; ■). Therefore, the data for the phasic responses at these 2 concentrations were combined for analysis. These results suggest that in addition to its role as a protonophore, CCCP enters the cell and interferes with an intracellular signaling step in sour taste transduction downstream of an acid-induced decrease in taste cell pH_i.

Relationship between pH_o, taste cell pH_i, and the CT response

Under control conditions, perfusing NaP or KP buffers produced a similar decrease in taste cell pH_i (data not shown). This is because the pK_a values of the NaP or KP buffers are the same. Under control conditions, KP buffers produced a significantly smaller decrease in taste cell pH_i (Figure 6A)

relative to post-nigericin treatment (150×10^{-6} M) (Figure 6B). However, after nigericin (150×10^{-6} M) treatment, the changes in taste cell pH_i induced by perfusing NaP buffers were not different from those shown in Figure 6A (data not shown). In 4 polarized taste bud preparations, the mean changes in taste cell pH_i before and after nigericin treatment for KP buffers are summarized in Figure 6C. In each case, we used the least squares fit second order polynomial displayed in Figure 6C to calculate a pH_i value corresponding to a given pH_o value.

A unilateral decrease in apical pH_o from 7.95 to 4.5 (ΔpH_o = 3.45) using KP or NaP buffers induced a mean decrease in taste cell pH_i from 7.32 to 7.04 (ΔpH_i = 0.28) and a ratio ($\Delta pH_i/\Delta pH_o$) of 0.08 (Figure 6C). In contrast, a unilateral decrease in the basolateral Ringer's solution pH from 7.8 to 6.8 (ΔpH_o = 1; Table 1) induced a sustained decrease in taste cell pH_i from 7.62 to 6.91 ($\Delta pH_i/\Delta pH_o$ = 0.71) (Supplementary Figure 3A). These results suggest that in taste cells the permeability of the apical membrane to H^+ is about 10-fold lower than that of the basolateral membrane (Lyall, Alam, Phan, Russell, et al. 2002). During calibration of the BCECF signal (F_{490}/F_{440}), bilaterally perfusing the apical and basolateral membranes with HK calibrating solutions of pH 8.0 and 6.5 containing 10×10^{-6} M nigericin (Table 1)
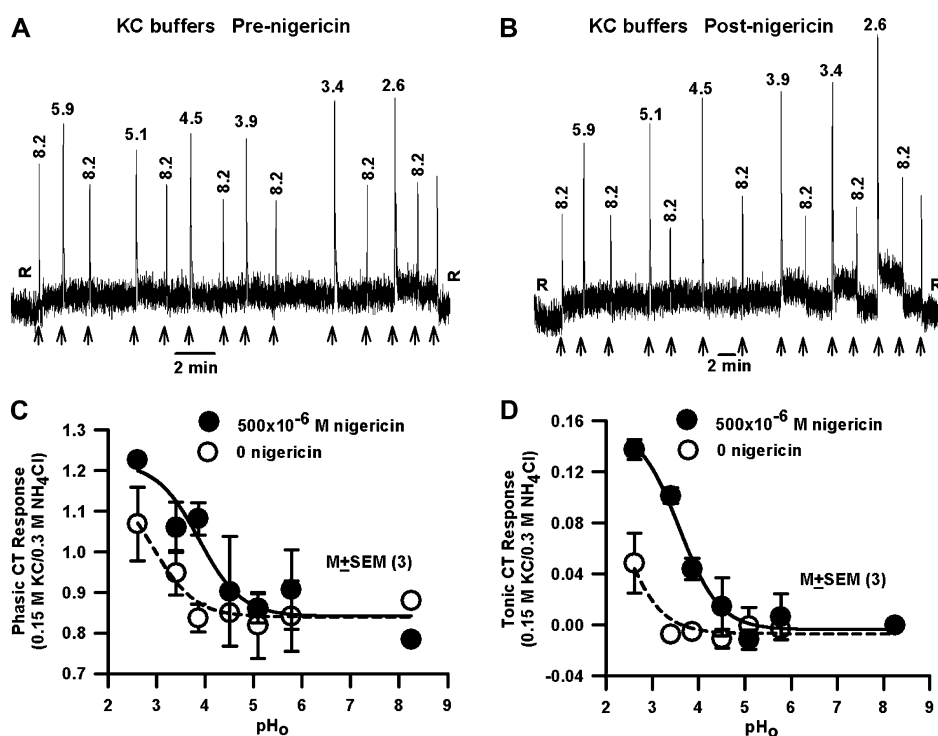
Figure 4 Effect of stimulating the tongue with potassium citrate (KC) buffered solutions on the rat CT response in the absence and presence of nigericin. KC buffers varying in pH between 8.0 and 2.9 were constituted by mixing various proportions of the 0.15 M potassium citrate and 0.15 M citric acid. Appropriate amount of additional KCl was added to different buffered solutions to keep K at 0.15 M. The tongue was first rinsed with the KC buffer at pH 8.2 and then with the KC buffers of pHs 5.9, 5.1, 4.5, 3.9, 3.4, and 2.6 **(A**; pre-nigericin**)**. Following this, nigericin (500×10^{-6} M dissolved in dimethyl sulfoxide, DMSO) was applied topically to the tongue for 30 min, and the CT responses were recorded as in its absence **(B**; post-nigericin**)**. The data from 3 animals showing the pH_o versus the normalized phasic **(C)** and tonic **(D)** CT response (0.15 M KC response/0.3 M NH_4Cl) is shown before (○) and after topical lingual application of 500×10^{-6} M nigericin (●). Each point represents the mean ± SEM phasic or tonic CT response from 3 animals.

produced near complete equilibration of pH_o and pH_i (Supplementary Figure 3B).

In the absence of nigericin, a decrease in taste cell pH_i evoked by NaP and KP buffers was not sufficient to elicit a phasic or tonic CT response (Figure 6D,E). Following nigericin treatment (150×10^{-6} M), KP buffers caused a decrease in taste cell pH_i sufficient to evoke phasic and tonic CT responses. If we assume threshold corresponds to a response of 5% of the maximum response, then for the phasic response (Figure 6D) the threshold value of pH_i would be 6.96. If threshold corresponds to a response 10% of maximum, then the threshold pH_i is 6.92. For the tonic response (Figure 6E), the 5% criterion gives a threshold pH_i of 6.91 and the 10% criterion gives a threshold pH_i of 6.87. In each case, therefore, threshold pH_i is, to a good approximation, 6.9.

Effect of nigericin and CCCP on CT responses to control stimuli

The normalized tonic CT responses to 0.3 M NH_4Cl and 0.3 M NaCl post-nigericin (Supplementary Figure 2A) and post-nigericin-post-CCCP (Supplementary Figure 2B) were not statistically different from their values after nigericin treatment alone ($P > 0.05$; paired). No effect of nigericin was observed on sweet (0.5 M sucrose, 0.25 M glycine or 0.005

M SC45647), bitter (0.01 M quinine), umami (0.1 M MSG + 5×10^{-6} M Bz + 1×10^{-6} M SB and MSG + Bz + SB + 0.001 M IMP) relative to control (data not shown). These results suggest that nigericin specifically alter CT responses to acidic stimuli without altering CT responses to salty, sweet, bitter, and umami stimuli. Consistent with the results shown in Figure 5, CCCP (500×10^{-6} M) inhibited CT responses to 0.02 M HCl. In addition, it also inhibited CT responses to 0.5 M sucrose and 0.02 M quinine (Supplementary Figure 2C).

Nigericin exchanges one K^+ (or Na^+) for one H^+ and is, therefore, electroneutral. The H^+ conductance through CCCP is electrogenic and is expected to depolarize the cell membrane potential (Kaila et al. 1989). Upon stimulation with acidic stimuli, the Ca^{2+} transient elicited by the decrease in pH_i in the subset of sour-sensitive taste cells is generated by Ca^{2+} influx through voltage-gated Ca^{2+} channels consequent to membrane depolarization (Roper 2007). We hypothesize that CCCP-induced cell depolarization inhibits the ΔpH_i-induced Ca^{2+} influx through voltage-gated Ca^{2+} channels and the CT responses to all acids. To test this hypothesis, we directly determined if changes in taste cell $[Ca^{2+}]_i$ are necessary to elicit CT responses to acidic stimuli.
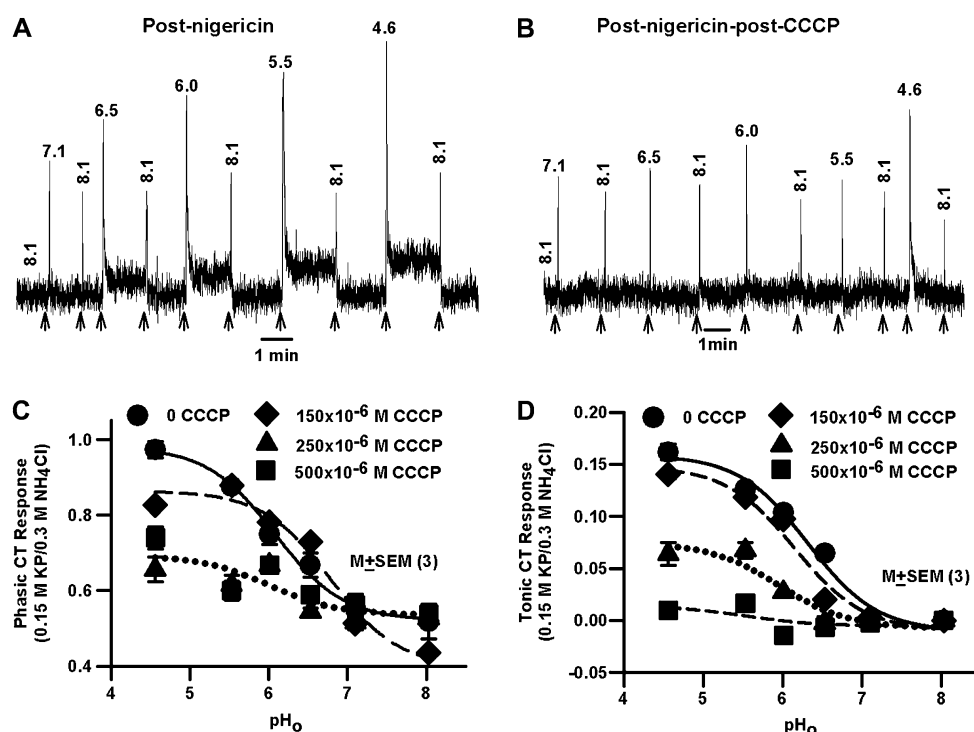
Figure 5 Effect of CCCP on the nigericin-induced changes in CT responses to KP buffers. **(A, B)** Figure shows a representative CT recording in which the rat tongue was initially treated with 500×10^{-6} M nigericin. CT responses to KP buffers of pHs 7.1, 6.6, 6.0, 5.3, and 4.6 were monitored relative to KP buffer of pH 8.1 **(A**; post-nigericin**)**. Following this, the tongue was treated with 500×10^{-6} M CCCP for additional 30 min, and the CT responses to KP buffers were monitored as before **(B**; post-nigericin-post-CCCP**)**. The relationships between pH_o and normalized phasic **(C)** and tonic **(D)** CT responses are shown as a function of CCCP concentration. Each point represents the normalized mean ± SEM phasic or tonic CT response (0.15 M KP CT response/0.3 M NH_4Cl) from 3 animals.

Effect of BAPTA and ionomycin on the CT responses to acidic stimuli

Fungiform taste bud cells were loaded with BAPTA in vivo by the topical application of 0.1×10^{-3}, 13×10^{-3}, and 33×10^{-3} M BAPTA-AM. BAPTA-AM is membrane permeable. Once inside the cell, it is hydrolyzed by intracellular nonspecific esterases and is converted to free acid. Intracellular BAPTA acid binds free $[Ca^{2+}]_i$ irrespective of its origin. Loading fungiform taste cells in vivo with BAPTA-AM (33×10^{-3} M) inhibited the tonic HCl CT response. The phasic component of the HCl CT response was not affected and is, therefore, Ca^{2+}-independent (Figure 7A) (Lyall et al. 2006). BAPTA-AM inhibited the tonic HCl CT response in a concentration-dependent manner (Figure 7B). To confirm that the tonic HCl CT response is dependent upon Ca^{2+} removal and is not a consequence of the intracellular BAPTA loading per se, we treated the taste bud cells in vivo with ionomycin (150×10^{-6} M) + Ca^{2+} (10×10^{-3} M). Following BAPTA loading, the lingual surface was superfused with the rinse solution (0.01 M KCl + 0.01 M $CaCl_2$) for 10 min to increase Ca^{2+} influx into taste cells and titrate all Ca^{2+}-binding sites on the intracellular BAPTA acid. After loading the taste cells with Ca^{2+} in the presence of ionomycin, the HCl tonic CT responses were not different from control (Figure 7B; post-BAPTA-post-iono + Ca^{2+} and Figure

7C). These studies provide direct evidence that in sour-sensing taste cells, ΔpH_i-induced increase in $[Ca^{2+}]_i$ is necessary for sour taste transduction (Lyall et al. 2004, 2006). No effect of BAPTA-AM was observed on the CT response to 0.3 M NH_4Cl or 0.1 M KCl (Figure 7C). We have previously shown that exposing the apical membrane to NH_4Cl acidifies taste cells (DeSimone et al. 2001). Unlike acid responses, the NH_4Cl CT responses are Ca^{2+} insensitive (Figure 7C). This suggests that NH_4Cl CT responses originate in a cell type different from the sour-sensing taste cells and that secondary changes in pH_i due to NH_3/NH_4^+ flux or in the presence of nigericin and CCCP do not affect CT responses to NH_4Cl (Supplementary Figure 2). Two NH_4^+ conduction pathways, a cellular pathway and a paracellular shunt pathway, mediate the transduction of NH_4 salts (Kloub et al. 1997). Thus, the use of NH_4Cl as a standard in these studies is valid.

Discussion

Acid entry via the K^+-H^+ exchanger nigericin

In polarized fungiform taste bud cells, a unilateral decrease in apical pH_o by 1 pH unit produced an approximately 10-fold lower change in taste cell pH_i relative to a unit decrease in basolateral pH_o (Figure 6A,C and Supplementary Figure 3A). This
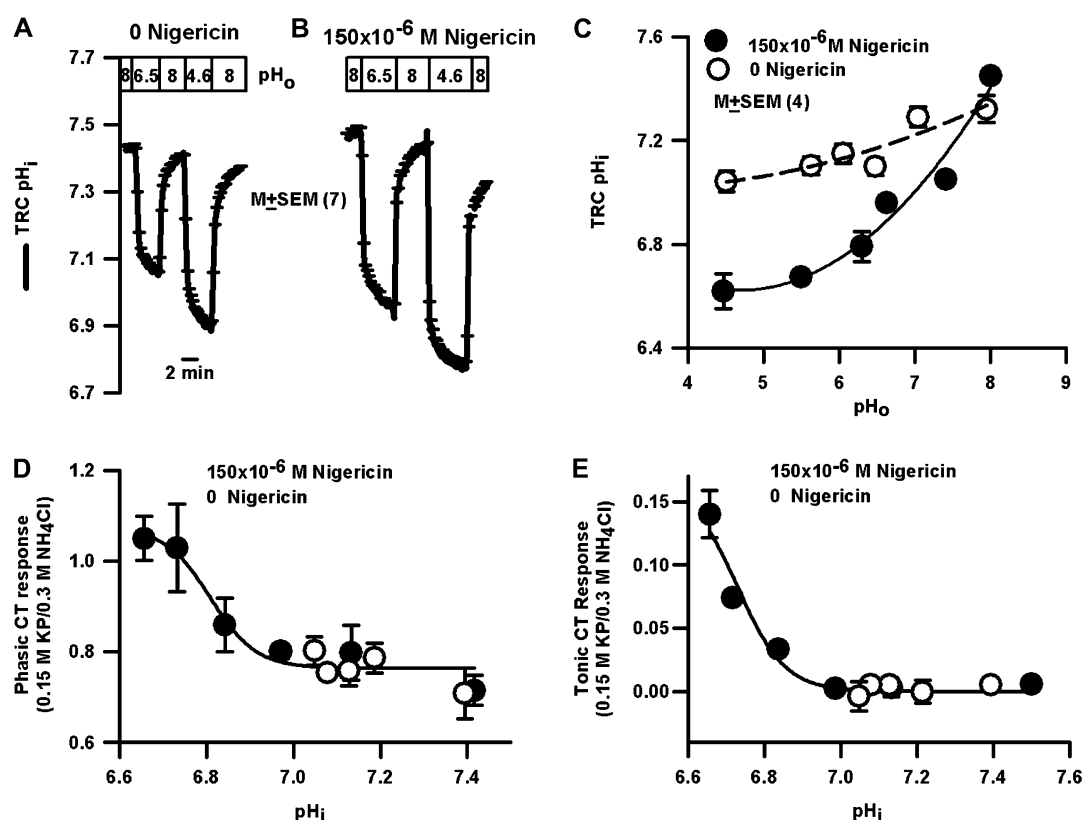
Figure 6   Relationship between pH_o, taste cell pH_i, and the CT response to KP buffers. The apical membrane of a polarized fungiform taste bud preparation loaded with BCECF was perfused with control Ringer's solution pH 7.4 (Table 1) containing 150×10^{-6} M nigericin for 20 min. Following this, the taste bud was perfused with KP buffers of pHs 8.0, 6.0, and 4.5 in the apical compartment while the basolateral compartment was continuously perfused with the control Ringer's solution at pH 7.4. **(A, B)** Figure shows representative temporal relationships between pH_o and taste cell pH_i and pH_o under control conditions (A; 0 nigericin) and after apical nigericin treatment (B; 150×10^{-6} M nigericin). Each time point represents the mean ± SEM value from 7 regions of interest taken within the taste bud. **(C)** Shows the relationship between pH_o and taste cell pH_i from the pooled data points obtained from 4 polarized fungiform taste bud preparations perfused with KP buffers before (0 nigericin; ○) and after treating the apical membrane with nigericin (150×10^{-6} M; ●). In each case, the curves are least squares fits of a second order polynomial to the data points as described in the Materials and methods section. **(D)** Shows the relationship between taste cell pH_i values calculated from the least squares curves in **(C)** using the appropriate experimental pH_o values and the magnitude of the phasic CT responses to KP buffers from Figure 2B before (0 nigericin; ○) and post-nigericin (150×10^{-6} M; ●). The solid curve is the least squares fit of a modified exponential in pH_i to the post-nigericin response points as described in the Materials and methods section. **(E)** Same as in (D) except that the tonic CT responses to KP buffers from Figure 2C before (0 nigericin; ○) and post-nigericin (150×10^{-6} M; ●) treatment is displayed.

suggests that both the apical cell membrane and the paracellular shunt pathway restrict H^+ entry, such that a large change in apical pH_o is translated into a relatively small change in taste cell pH_i that remains within the physiological range (Lyall et al. 2001; Lyall, Alam, Phan, Phan, et al. 2002; Lyall, Alam, Phan, Russell, et al. 2002). Incorporating nigericin in the apical membrane of taste cells provides an acid entry mechanism via the K^+-H^+ exchanger. In the presence of nigericin (150×10^{-6} M), unilaterally superfusing the apical membrane of taste cells with same KP buffers induced a 2.9-fold larger decrease in taste cell pH_i (Figure 6B,C). At high nigericin concentration (500×10^{-6} M), H^+ entry via the K^+-H^+ exchanger induced by KP buffers was comparable to acid entry via proton channels and/or via passive diffusion as the undissociated acid (Figure 3C).

Although the ionophore selectivity of nigericin toward K^+ is 25–45 times higher than that toward Na^+, the selectivity of nigericin for K^+ over Na^+ and its intrinsic rate constants for the translocation of nigericin-H^+ and for the translocation of nigericin-Na^+ or nigericin-K^+ across the membrane depend upon the pH and the concentration of Na^+ and K^+ (Prabhananda and Ugrankar 1991). In the absence of external Na^+ or K^+, nigericin also enhanced the CT response to HCl (Figure 3D). Under these conditions, the exit of Na^+ and K^+ across the apical membrane of taste cells is exchanged for H^+ entry via nigericin. Because the K^+ gradient across the apical membrane is greater than the Na^+ gradient (in > out), it suggests that in the absence of external ions, the K^+-H^+ exchange via nigericin plays a significant role in H^+ entry. We have previously shown that stimulating the apical membrane of polarized fungiform taste cells with unbuffered Ringer's solution containing 1×10^{-3} M (pH_o 3.0), 10×10^{-3} M (pH_o 2.0), and 30×10^{-3} M (pH_o 1.5) HCl decreased mean taste cell pH_i by 0.27 ± 0.03, 0.36 ± 0.03, and 0.59 ± 0.02
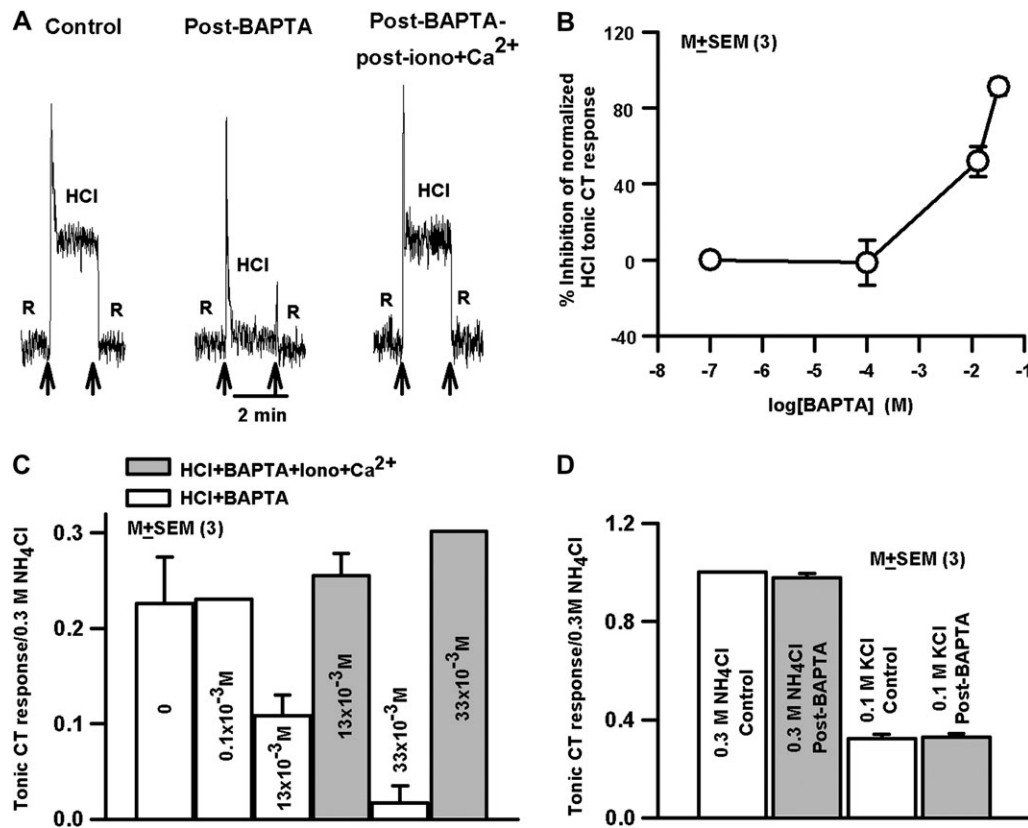
Figure 7   Effect of BAPTA and ionomycin on the rat CT responses to HCl. **(A)** Figure shows a representative CT recording in which the rat tongue was first rinsed with a rinse solution R (0.01 M KCl) and then with 0.02 M HCl under control conditions (Control); after topical lingual application of 33×10^{-3} M BAPTA-AM (post-BAPTA) and post-BAPTA topical lingual application of 150×10^{-6} M ionomycin (iono) + 0.01 M CaCl$_2$ (post-BAPTA-post-iono + Ca^{2+}). The arrows represent the time period when the tongue was superfused with rinse or HCl solutions. **(B)** CT responses to 0.02 M HCl were monitored with reference to 0.01 M KCl rinse after treating the apical membrane with 0, 0.1×10^{-3}, 13×10^{-3}, and 33×10^{-3} M BAPTA-AM. At each BAPTA concentration, the CT response to HCl was normalized to the response to 0.3 M NH$_4$Cl response and was calculated with reference to its magnitude in the absence of BAPTA-AM. The data are presented as % inhibition of the normalized HCl tonic CT response relative to CT response in the absence of BAPTA-AM. **(C)** Shows the mean ± SEM normalized tonic HCl CT response in 3 rats at different BAPTA-AM concentrations before and after topical lingual application of 150×10^{-6} M ionomycin + 0.01 M CaCl$_2$. **(D)** Shows the effect of BAPTA-AM on mean normalized CT responses to 0.3 M NH$_4$Cl and 0.1 M KCl before and after topical lingual application of 33×10^{-3} M BAPTA-AM. The CT responses were normalized to the NH$_4$Cl response at the beginning of the experiment.

(SEM) pH unit, respectively. The pH$_o$ versus the normalized tonic CT responses and the normalized changes in taste cell pH$_i$ in vitro demonstrated similar profiles (Lyall, Alam, Phan, Phan, et al. 2002). These results suggest that nigericin increases the HCl CT response by further lowering the taste cell pH$_i$.

In contrast, post-nigericin treatment, NaP buffers did not produce the same effect on CT responses as observed with KP buffers (Figure 2A,B). In presence of NaP buffers (150 mM Na$^+$), Na$^+$ entry via nigericin is exchanged for H$^+$ exit and is expected to cause intracellular alkalinization. On the other hand, the K$^+$ exit from the cells via nigericin (0 K$^+$ outside) is exchanged for H$^+$ entry and is expected to cause intracellular acidification. Because, in our studies, NaP buffers induced a similar decrease in taste cell pH$_i$ in the absence and presence of 150×10^{-6} M nigericin (data not shown), we hypothesize that in the presence of apical NaP buffers the H$^+$ exit coupled to Na$^+$ entry is compensated

by the H$^+$ entry coupled to K$^+$ exit. This is possible because in the presence of NaP buffers, the outward K$^+$ gradient across the apical membrane of taste cells is greater than the inward Na$^+$ gradient.

In synaptosomes, at physiological Na$^+$ concentration nigericin increased intracellular Na$^+$ ([Na$^+$]$_i$) and increased pH$_i$. In the absence of a Na$^+$ concentration gradient (i.e., when the external Na$^+$ concentration equals the [Na$^+$]$_i$), nigericin causes the opposite effect on the pH$_i$ without altering [Na$^+$]$_i$. These results suggest that under physiological conditions nigericin behaves as a Na$^+$-H$^+$ ionophore. It allows entrance of Na$^+$ in exchange for H$^+$ through the ionophore itself. Nigericin behaves as a K$^+$-H$^+$ ionophore in synaptosomes when the net Na$^+$ movement is eliminated (i.e., under conditions in which the external and the internal Na$^+$ concentrations are equal) (Rodríguez and Sitges 1996). Both in our CT recordings and pH$_i$ studies, the apical lingual surface was first adapted to NaP or KP buffered solution of

pH 8.0 for 10 min. The subsequent changes in CT response or a decrease in taste cell pH_i was measured as a response to a decrease in pH_o without a significant change in Na^+ or K^+ concentration in the apical compartment (Table 1). Under constant external Na^+ concentration (0.15 M Na^+ in NP buffers and 0 Na^+ in KP buffers), the net Na^+ movement across the apical membrane remains unchanged and nigericin seems to behave as a K^+-H^+ ionophore (Figure 6A,B). Although the presence of the Na^+-H^+ exchanger-3 (NHE-3) was observed in the apical membrane of taste cells with NHE-3 antibodies, changes in Na^+ concentration in the apical compartment of polarized fungiform taste cells did not induce changes in taste cell pH_i (Vinnikova et al. 2004). This suggests that apical NHE-3 is not active and does not contribute to changes in taste cell pH_i in the presence or absence of Na^+ in NP and KP buffers and in the absence or presence of nigericin treatment.

In the presence of nigericin, acid entry is regulated by varying the concentration of nigericin incorporated in the apical membrane and by the pH of KP buffers (Figure 2). Because under our experimental conditions, nigericin does not appear to exchange Na^+ with H^+, it provides its own control for acid entry using NaP buffers (Figure 2A). It is important to note that at 500×10^{-6} M nigericin, distinct phasic and tonic CT responses could be observed by stimulating the tongue with a KP buffer of pH 6.6. The observation that a KP buffer, at a pH of 3 to 4 units above those of unbuffered acids, yields CT responses comparable to those of the acids (Figure 3C) is strong evidence that CT responses to acids are largely indifferent to pH_o per se. In the presence of nigericin, K^+-H^+ exchange is the primary mode of acid entry when taste cells are superfused with KP buffers. Acid entry via exchanger-independent mechanisms does not contribute to the CT response over the pH range of the KP buffers used in these experiments. This is additional supportive evidence that CT responses to acidic stimuli are independent of the pH_o of the acidic stimulus. The effects of nigericin were specific to acidic stimuli. Nigericin produced no effect on the CT response to salts (NaCl or NH_4Cl; Supplementary Figure 2) and to sweet, bitter, or umami stimuli (data not shown).

Phasic and tonic CT responses to KP buffers demonstrate a strict dependence upon the acid-induced decrease in TRC pH_i

In our studies, CT responses and changes in taste cell pH_i were measured at the same nigericin concentration. In our in vitro polarized fungiform taste bud preparations, the changes in pH_i were observed within the same time frame as our CT recordings. In polarized taste cells, decreasing the pH_o of KP or NaP buffers induced a decrease pH_i (Figure 6A–C). This decrease in taste cell pH_i does not seem to be sufficient to excite the CT nerve (Figure 6D,E). In the presence of apical nigericin, the same KP buffers induced a significantly greater intra-

cellular acidification (Figure 6B,C). Using both a 5% and 10% of maximum response criterion yields, for both phasic and tonic responses as a function of pH_i (Figure 6D,E), an estimate of about 6.9 as the threshold value of pH_i post-nigericin (150×10^{-6} M). Although this number cannot be determined precisely, the threshold pH_i value must be less than approximately 7.0, the lowest value achieved with KP buffers in the absence of nigericin, and for which no CT response was observed. In this study, the 5% or 10% of the maximum CT response was arbitrarily chosen to estimate the threshold CT response. In general, using a lower threshold value for the CT response will shift the threshold value to a slightly more alkaline pH.

It is important to note that post-nigericin, KP buffers elicited CT responses between pH 6.6 and 4.6. However, the relationship between the magnitude of the CT response and pH_i was observed over a very narrow taste cell pH_i range (Figure 6D,E). Thus, the decrease in pH_i during sour taste transduction occurs within 0.3 to 0.4 pH units below the resting taste cell pH_i, as is the case for other acid-sensitive chemosensory cells (DeSimone and Lyall 2006). In the presence of 150×10^{-6} M nigericin, the decrease in taste cell pH_i induced by KP buffers was small, and complete equilibrium between pH_o and pH_i could not be achieved during the time frame of our experiments (Figure 6C). In contrast, under control conditions, taste cell pH_i tracked changes in basolateral pH_o with a slope of 0.7 (Supplementary Figure 3A). This close relationship between basolateral pH_o and taste cell pH_i is due to the high-proton permeability of the basolateral membrane, depolarization of the basolateral membrane potential in the presence of high K^+, and inhibition of the basolateral NHE-1 at low pH_o. During pH_i calibration, nigericin and high K^+ calibrating buffers were applied to both apical and basolateral membranes (Table 1). Under these conditions, within a few minutes, complete equilibrium is achieved between pH_o and pH_i (Supplementary Figure 3B). Thus, in the presence of apical nigericin, KP buffers will decrease taste cell pH_i to a value that reflects a nonequilibrium steady state that depends on the rate of H^+ entry via the K^+-H^+ exchanger, the amount of intracellular buffering and the rate of H^+ exit across the basolateral membrane (Lyall et al. 2001; Lyall, Alam, Phan, Phan, et al. 2002).

The phasic CT response has 2 components. One component is derived from the transient mechanical rinse artifact that is observed every time the lingual surface is superfused with a rinse or test solution (Figures 1A–C, 4A, and 5B). The second component constitutes the chemical response of the nerve to taste stimuli applied to the tongue. The phasic response is sensitive to the rate at which the taste stimulus is superfused in the lingual chamber (Lyall et al. 2001). In contrast, the tonic component of the CT response is stable and is not affected by the rate of superfusion and the mechanical rinse artifact. Consistent with this, under control conditions, CT responses to KP and NaP buffers elicited only the mechanical rinse artifact (Figure 1A). Post-nigericin, the same

KP buffers elicited a phasic response that was significantly larger than the rinse artifact (Figure 1B).

In our studies, CCCP inhibited CT responses to KP buffers post-nigericin in a concentration manner (Figure 5). CCCP also inhibited CT responses to HCl, sucrose, and quinine (Supplementary Figure 2C). Proton conductive flux via CCCP is expected to depolarize taste cells (Kaila et al. 1989). The changes in cell membrane potential can affect both the phasic and tonic component of the CT response to acidic stimuli. We have previously shown that a decrease in pH_i induces a change in cell cytoskeleton through a shift in the F-actin to G-actin equilibrium that is involved in isosmotic cell shrinkage. The phasic component of the CT response to acid stimulation is derived from cell shrinkage-induced activation of flufenamic acid-sensitive nonspecific cation channels in the basolateral membrane of taste cells that allow cations to enter the cell to cause depolarization (Lyall et al. 2006). The phasic CT response to HCl is independent of Ca^{2+} (Figure 7A). In contrast, the tonic phase of the HCl CT response is Ca^{2+} dependent. An increase in acid-induced increase in taste cell $[Ca^{2+}]_i$ is necessary for the elicitation of the tonic response to acidic stimuli (Figure 7A). It is suggested that the Ca^{2+} transient elicited by ΔpH_i in the subset of sour-sensitive cells is generated by Ca^{2+} influx through voltage-gated calcium channels consequent to membrane depolarization (Richter et al. 2003). It is likely that a proton-gated ion channel or a volume-sensitive ion channel is involved in depolarizing the receptor potential in sour-sensing cells that leads to an increase in $[Ca^{2+}]_i$ and the generation of tonic CT response to acidic stimuli. The increase in $[Ca^{2+}]_i$ activates basolateral NHE-1 that is involved in pH_i and cell volume recovery during sour taste transduction and determines the level of adaptation in the neural response (Lyall et al. 2004, 2006). In Type III acid sensing taste cells, Ca^{2+} influx is necessary for vesicular neurotransmitter release (Roper 2007; Medler 2010).

In contrast, in Type II sweet, bitter, and umami sensing cells, taste specific G protein–coupled receptors initiate a signaling cascade that activates the phospholipase C beta-2 isoform of the enzyme ($PLC\beta_2$) to generate inositol 1,4,5-trisphosphate (IP3) that causes Ca^{2+} release from internal Ca^{2+} stores. An increase in $[Ca^{2+}]_i$ activates TRPM5 on the basolateral membrane of taste cells and causes depolarization of the receptor potential via an influx of monovalent cations (Huang et al. 2006). Type II cells lack conventional synapses and rely on the opening of a hemichannel to release ATP as a neurotransmitter (Huang et al. 2007; Roper 2007). It was suggested that only Type II cells release ATP and only Type III presynaptic cells release serotonin (Huang et al. 2007). However, ATP seems to be crucial for communication from taste buds to gustatory nerves for all taste qualities (Finger et al. 2005). We hypothesize that CCCP inhibits the sweet and bitter taste transduction mechanism by irreversibly depolarizing and thereby desensitizing the receptor cell.

In summary, the relationship between taste cell pH_i and the CT response obtained using KP buffers, after apical nigericin incorporation, reinforces the hypothesis that acid-induced decrease in taste cell pH_i is the proximate signal for sour taste transduction as proposed earlier (Lyall et al. 2001, 2004, 2006; Lyall, Alam, Phan, Phan, et al. 2002; Lyall, Alam, Phan, Russell, et al. 2002).

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/

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