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Responses of the Hamster Chorda Tympani Nerve to Sucrose+Acid and Sucrose+Citrate Taste Mixtures

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Abstract

Studies of taste receptor cells, chorda tympani (CT) neurons, and brainstem neurons show stimulus interactions in the form of inhibition or enhancement of the effectiveness of sucrose when mixed with acids or citrate salts, respectively. To investigate further the effects of acids and the trivalent citrate anion on sucrose responses in hamsters (*Mesocricetus auratus*), we recorded multifiber CT responses to 100 mM sucrose; a concentration series of HCl, citric acid, acetic acid, sodium citrate (with and without amiloride added), potassium citrate, and all binary combinations of acids and salts with 100 mM sucrose. Compared with response additivity, sucrose responses were increasingly suppressed in acid + sucrose mixtures with increases in titratable acidity, but HCl and citric acid were more effective suppressors than acetic acid. Citrate salts suppressed sucrose responses and baseline CT neural activity to a similar degree. Citrate salts also elicited prolonged, concentration-dependent, water-rinse responses. The specific loss in sucrose effectiveness as a CT stimulus with increasing titratable acidity was confirmed; however, no increase in sucrose effectiveness was found with the addition of citrate. Further study is needed to define the chemical basis for effects of acids and salts in taste mixtures.

Key words: acid, chorda tympani, citrate salts, sucrose, taste mixtures, water-rinse responses

Introduction

Mixture interactions in mammalian taste, inhibitory or synergistic, occur in the peripheral and central gustatory systems (Hyman and Frank 1980a, 1980b; Travers and Smith 1984; Yamamoto et al. 1991; Vogt and Smith 1993, 1994; Formaker and Frank 1996; Miyaoka and Pritchard 1996; Formaker et al. 1997; Stapleton et al. 2000; Sako et al. 2003; Chen and Di Lorenzo 2008). For example, in the golden hamster (*Mesocricetus auratus*), NaCl suppresses responses to quinine in the chorda tympani (CT) (Formaker and Frank 1996) and quinine suppresses responses to sucrose in the CT and parabrachial nucleus (PbN) (Formaker and Frank 1996; Formaker et al. 1997; Vogt and Smith 1994). Sucrose-best neurons of the hamster CT (Hyman and Frank 1980b) and hamster PbN neurons (Travers and Smith 1984) show response suppression to mixtures of HCl with sucrose. Hamster PbN neurons also show response suppression to mixtures of citric acid with sucrose (Vogt and Smith 1993). Thus, acids appear to suppress electrophysiological taste responses to sucrose, and suppression of whole-nerve responses to sucrose + acid mixtures may reflect reduced activity in the sucrose-best subset of hamster CT neurons.

Although citric acid is known to suppress responses to sucrose in the central gustatory system (Vogt and Smith 1993), it is not known whether this mixture interaction originates from the peripheral or central gustatory system. It is possible that acid–sucrose response suppression originates in the peripheral gustatory system. The use of citric acid is also important because rat geniculate ganglion cell responses to mineral acids (Lundy and Contreras 1999) are distributed more generally across electrolyte generalist CT fibers than responses to carboxylic acids (Breza et al. 2008). Whether the narrow carboxylic acid–response distribution impacts upon responses to acid + sucrose mixtures in the peripheral gustatory system is unknown. Thus, the present study used two carboxylic acids (citric acid and acetic acid) and a mineral acid (HCl) in mixtures with sucrose to assess the potential effects of acids on sucrose taste responses in the peripheral gustatory system.

The present study also used citrate salts in mixtures with sucrose. In contrast to acid's suppression, trivalent citrate ions have been reported to enhance behavioral taste preferences and responses of taste receptor cells (TRCs) to sucrose

in the rat (Gilbertson et al. 1997). However, effects of the citrate anion on responses of rat and hamster CT nerves are currently unknown.

The present study used acids and salts as taste stimuli to examine the effects of acidity and the trivalent citrate anion on taste responses to mixtures with sucrose in the hamster CT. Citric, acetic, and hydrochloric acid were each combined with sucrose to determine the effects of H^+ on sucrose responses. Trisodium and tripotassium citrate salts were combined with sucrose to determine the effects of the citrate anion on sucrose responses. It was hypothesized that acidic stimuli would suppress hamster CT responses to sucrose and the nonacidic citrate salts would enhance hamster CT responses to sucrose.

Materials and methods

Subjects and surgical procedure

Whole-nerve electrophysiological taste responses were recorded from the right CT nerve of 28 adult, male, golden hamsters (105–160 g). Hamsters were anesthetized by intraperitoneal injection of sodium pentobarbital (initial dose: 80 mg/kg; subsequent dosage to maintain a surgical level of anesthesia: 40 mg/kg). Body temperature was regulated at ~37 °C with a Deltaphase isothermal pad. A tracheal cannula was implanted to assist breathing, and the hypoglossal nerve was transected bilaterally to prevent inadvertent tongue movements. The right CT nerve was exposed using a mandibular approach. The CT was cut near its entrance to the tympanic bulla, desheathed, and placed on a nichrome wire recording electrode with an indifferent electrode placed in nearby tissue. Protocols were approved by the animal care committee of the University of Connecticut Health Center in Farmington, CT.

Electrophysiology

Multifiber neural activity was differentially amplified, square rectified, and summed (200-ms time constant) before digitization with a Cambridge Electronic Design (CED) Micro 1401 II analog to digital converter. Digitized data were displayed (see, e.g., Figures 1 and 4) and saved on a PC for offline analysis using CED's *Spike2* software.

Gustatory stimulation

Taste stimuli were presented to the anterior region of the tongue via a gravity flow system at a rate of ~2 mL/s. Stimuli were presented for ~15 s followed by a distilled water rinse for at least 45 s. Stimuli were presented at room temperature (~21 °C). Binary mixtures were prepared such that component concentrations of each mixture equaled the concentrations of each component presented alone. The single components of every binary mixture, along with the mixture itself, were separately applied to the tongue for every mixture in the study. Mixtures were presented in ascending

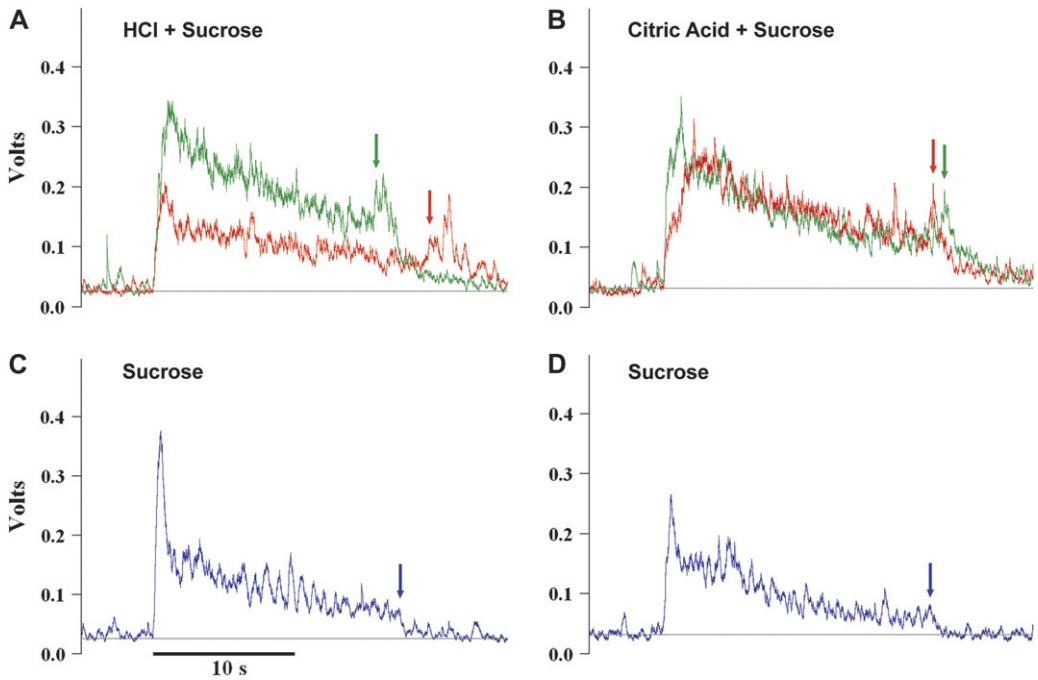

Figure 1 Integrated multifiber CT responses to anterior tongue stimulation with single components and binary mixtures of sucrose with HCl or citric acid. **(A)** Component response to 3 mM HCl alone (smaller, red trace) superimposed on the mixture response to 3 mM HCl + 100 mM sucrose (larger, green trace). **(B)** Component response to 10 mM citric acid alone (red trace) superimposed on the mixture response to 10 mM citric acid + 100 mM sucrose (green trace). **(C, D)** Component responses to sucrose alone, beneath each mixture, respectively. Dotted lines = average baseline voltage; downward arrows = water rinses.

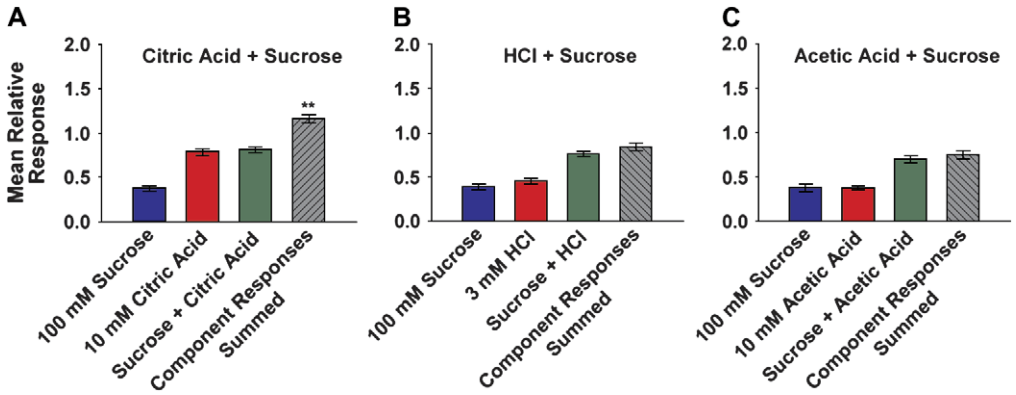

Figure 2 Mean (±standard error of the mean) relative CT responses to each of the 3 acids used in phase 1, **(A)** 10 mM citric acid, **(B)** 3 mM HCl, and **(C)** 10 mM acetic acid. Component responses summed were obtained by adding the responses to each of the mixture components presented separately. The response to the sucrose + citric acid mixture was smaller than that predicted by the component responses summed implying sucrose-response suppression. In contrast, responses to sucrose + HCl or sucrose + acetic acid were similar to the component responses summed implying modality independent responses; $^{**}P < 0.001$.

concentration order, and 500 mM NH_4Cl was applied at the beginning and end of each binary mixture series. For example, 500 mM NH_4Cl was presented first, followed by the two individual component stimuli, the binary mixture and, finally, 500 mM NH_4Cl again. The sequence would then repeat with the next concentration of acid or salt. Consequently, 100 mM sucrose was applied several times as a single component of each binary mixture across the various mixture applications. The order of stimuli presented between NH_4Cl presentations varied. The NH_4Cl solution was used as a reference stimulus in the data analyses and to monitor preparation stability over time.

Experimental phases

The experiment was divided into three phases. "Phase 1" taste stimuli were 3 mM hydrochloric acid (HCl, n = 14), 10 mM citric acid (n = 15), 10 mM acetic acid (n = 12), 100 mM sucrose, and the binary combinations of each acid with 100 mM sucrose. The n for sucrose corresponded with the n for acid in each mixture series. N differs because all acid stimulus presentations were not completed on every animal. Each acid + sucrose mixture was analyzed separately as a within subjects design. Two of the acids, HCl and citric acid, were pH matched and two, citric acid and acetic acid, were concentration matched. "Phase 2" stimuli expanded upon Phase 1 and consisted of a concentration series of citric acid (1, 3, and 10 mM, n = 10), HCl (1, 3, and 10 mM, n = 6), acetic acid (10, 30, and 100 mM, n = 7), and the binary combinations of each acid with 100 mM sucrose. Finally, "Phase 3" stimuli did not include the acids but, instead, were a concentration series (1, 3, and 10 mM) of potassium citrate, sodium citrate, sodium citrate mixed in a solution of 30 μM amiloride, and binary combinations of these citrate salts with 100 mM sucrose (n = 8). Amiloride blocks the responses of Na^+-best CT neurons associated with the epithelial sodium channel (ENaC) (Ninomiya and Funakoshi 1988; Hettinger and Frank 1990; Shigemura et al. 2008). Thus, Phases 1 and 2 addressed acidity and Phase 3 citrate effects on CT responses to mixtures with sucrose. Table 1 lists the measured pH of the acidic stimuli.

Data analysis

Taste-stimulated responses were quantified as the 5-s area under each integrated stimulus-response curve beginning with stimulus onset. The 5-s area measured overall CT neural activity for the initial 5 s of stimulus application. CT activity elicited by rinses following citrate salts was quantified as the 30-s area under the integrated rinse response curve beginning with rinse onset. This measured the overall CT neural activity of the very lengthy rinse responses. All responses were expressed relative to the mean of the two standard NH_4Cl responses bracketing each mixture series, and data are presented as means ± standard errors (standard error of the mean). Relative response magnitudes for each binary mixture and the water-rinse responses were analyzed using repeated-measures ANOVA. Post hoc analyses used the Tukey test to control Type I error (Seaman et al. 1991). Responses to sucrose + acid or sucrose + salt mixtures should equal the sum of the responses to each component presented separately (i.e., response additivity), if the mixture components have completely independent effects on the peripheral gustatory system. Mixture suppression or enhancement can only occur if the mixture components interact. Greater percent suppression or enhancement implies greater interaction among the mixture components. Percent sucrose-response suppression was calculated as: [1 – ((mixture response – acid or salt component response) ÷ sucrose component response)] × 100. For example, in phase 1, the average relative response to 10 mM citric acid was 0.78, 100 mM sucrose: 0.38, and the citric acid + sucrose mixture: 0.79. Substituting these values into the percent suppression equation, [1 – ((0.79 – 0.78) ÷ 0.38)] × 100 = 97% sucrose-response suppression.

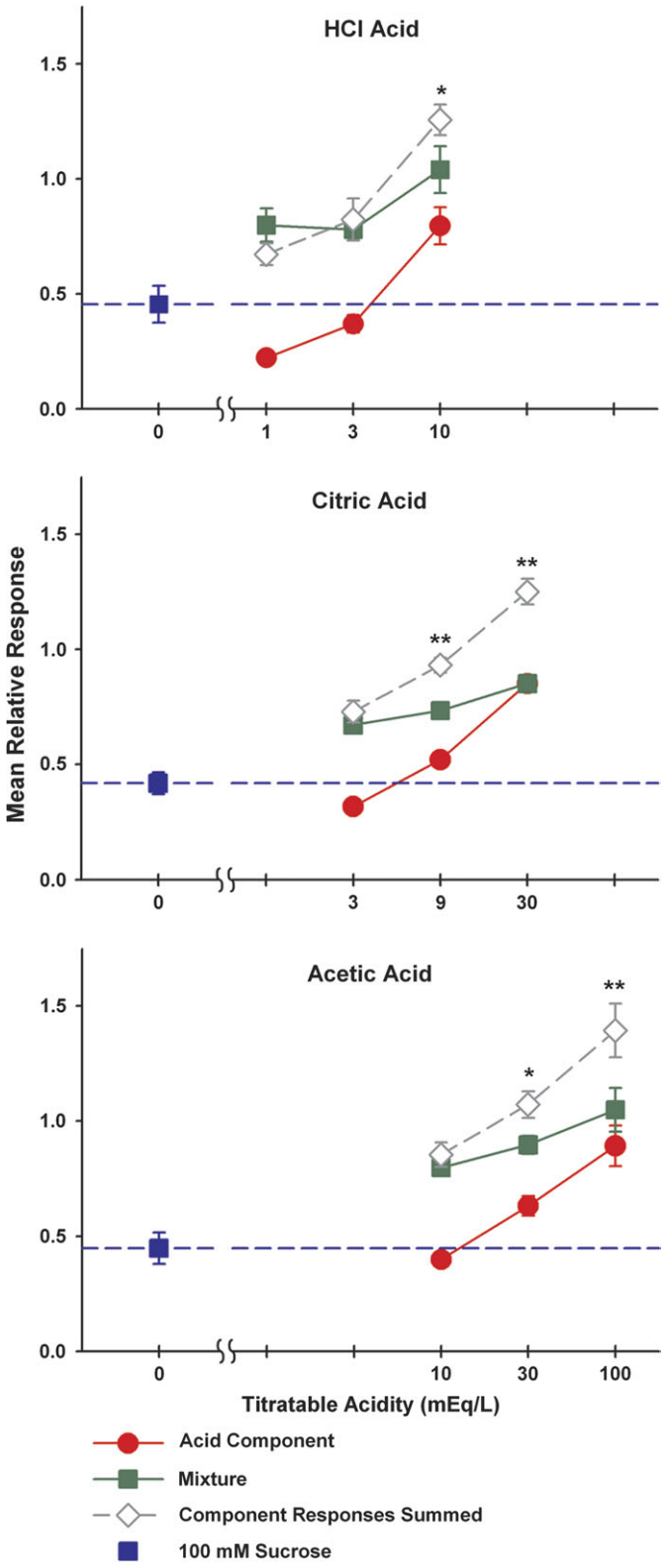

Figure 3 Mean (±standard error of the mean) CT responses to acid + sucrose mixtures (squares) plotted as a function of titratable acidity. As titratable acidity increased, responses to each of the sucrose + acid mixtures deviated from the component responses summed (open diamonds). Filled circles illustrate responses to the acid components alone. Single square, with horizontal dashed line, shows the average response to 100 mM sucrose for each acid + sucrose mixture. These results indicate a suppressive interaction between the acidic stimuli and sucrose. (*$P < 0.05$, **$P < 0.01$, mixture response vs. component responses summed.)

Results

Phase 1

Responses to mixtures of 100 mM sucrose with 3 mM HCl or 10 mM acetic acid were additive; however, responses to a mixture of sucrose and 10 mM citric acid were less than additive ($P < 0.001$). Thus, the two acids matched for pH (2.5), 3 mM HCl and 10 mM citric acid, and the two acids matched for concentration, 10 mM acetic acid and citric acid, had dissimilar effects on CT responses to sucrose in binary acid + sucrose mixtures. Figures 1 and 2 illustrate these results. Responses to HCl + sucrose and acetic acid + sucrose mixtures, each larger than the responses to either mixture component presented separately, were equivalent to the component responses added together. In contrast, responses to the citric acid + sucrose mixture were the same size as the citric acid response itself, implying suppression of CT sucrose responses. These results suggest that titratable acidity >10 mEq/L may be required for suppressing sucrose responses. Titratable acidities, in mEq/L, for the acids used in Phase 1 were 3 for HCl, 10 for acetic acid, and 30 for citric acid.

Phase 2

Figure 3 graphs the responses to sucrose + acid mixtures plotted against titratable acidity. At ~10 mEq/L acidity, HCl and citric acid suppressed responses to sucrose; at 30 mEq/L, acetic acid suppressed responses to sucrose. Individual responses to the HCl, citric acid, and acetic acid components all increased with titratable acidity ($P < 0.05$; Figure 3A,B,C). Examination of the significant stimulus by titratable acidity interaction for the sucrose + HCl mixture series, $F(6,30) = 12.28$, $P < 0.0001$, revealed that mixture responses were statistically equivalent to the component responses added together at 1 and 3 mEq/L, but "less than additive" at 10 mEq/L ($P < 0.05$). The stimulus by titratable acidity interaction for sucrose + citric acid mixtures, $F(6,54) = 46.22$, $P < 0.00001$, revealed that mixture responses were additive at 3 mEq/L, but less than additive at 9 and 30 mEq/L ($P < 0.001$). Finally, the stimulus by titratable acidity interaction for sucrose + acetic acid mixtures, $F(6,36) = 13.19$, $P < 0.00001$, revealed that mixture responses were additive at 10 mEq/L, but less than additive at 30 ($P < 0.05$) and 100 mEq/L ($P < .001$). Thus, mixture suppression increased as a function of titratable acidity, a measure of total available H^+. However, sucrose-response suppression began above 3 mEq for citric acid and HCl but above 10 mEq for acetic acid, the weakest of the 3 acids.

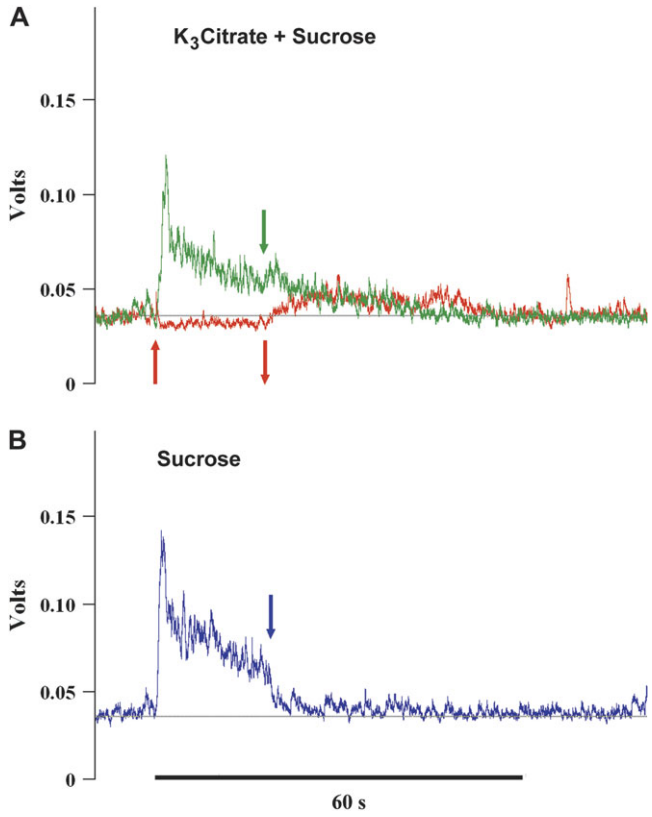

Figure 4 **(A)** Integrated multifiber CT responses to anterior tongue stimulation with 1 mM K_3Citrate alone (smaller, red trace) superimposed on the response to a mixture of 1 mM K_3Citrate + 100 mM sucrose (larger, green trace). K_3Citrate had a small inhibitory effect on the CT; release of K_3Citrate inhibition is evident in the prolonged water-rinse response. **(B)** The response to the 100 mM sucrose component presented alone. Dotted lines = average baseline voltage; upward arrow = stimulus onset; downward arrows = water rinse.

Table 1 Measured pH[a]

Acid	pH
1 mM HCl	3.0
3 mM HCl	2.5
10 mM HCl	2.0
10 mM acetic acid	3.2
30 mM acetic acid	2.9
100 mM acetic acid	2.7
1 mM citric acid	3.0
3 mM citric acid	2.7
10 mM citric acid	2.5

[a]Adding sucrose did not change the pH of acid-containing stimuli, and the pH of all salts were ≥5.

Phase 3

Figure 4 shows representative raw data tracings of hamster CT responses to 1 mM K_3Citrate, 100 mM sucrose, and a mixture of 1 mM K_3Citrate with 100 mM sucrose. Note the neural response decrement to K_3Citrate and the slow water-rinse responses following K_3Citrate and the sucrose + K_3Citrate mixture. K_3Citrate and Na_3Citrate mixed with amiloride, both as single components presented separately and in binary mixtures with sucrose, had similar effects on CT neural activity. Because responses to K_3Citrate and Na_3Citrate mixed with amiloride were statistically equivalent, responses to stimuli containing these two salts were averaged together and are illustrated combined together in Figure 5A as, "citrate salts." Overall, 30 μM amiloride reduced Na_3Citrate responses by 92% and mixture responses by 47%. Figure 5A shows that the average responses to 100 mM sucrose mixed with 1 and 3 mM K_3Citrate or Na_3Citrate mixed with amiloride were ~15% smaller than the average response to sucrose alone ($P < 0.05$). Although not reliably different from baseline activity (i.e., zero), measured responses to 1 and 3 mM K_3Citrate alone were –0.019 ± 0.01 and –0.005 ± 0.02, respectively (data not shown), suggesting the inhibition of CT activity. Figure 5B shows the effects of Na_3Citrate and Na_3Citrate + sucrose mixtures without amiloride inhibition. Responses to mixtures of sucrose and Na_3Citrate were additive, except at 10 mM Na_3-Citrate, where the mixture response was still greater than either of the two components presented alone but less than the response predicted by adding the component responses together ($P < 0.01$).

Interestingly, deionized water rinses were effective CT stimuli immediately following K_3Citrate, Na_3Citrate, or sucrose + citrate mixtures. Rinse responses, seen in Figure 4, are quantified in Figure 6. Rinse responses were analyzed using a 3-factor, within-subjects, ANOVA with "stimulus" (K_3Citrate, Na_3Citrate, and Na_3Citrate plus amiloride), "concentration" (1, 3, and 10 mM), and "mixture" (with sucrose or without sucrose) as the 3 within-subjects factors. None of the interaction terms were significant; therefore, focus was on the main effects. Analysis of the concentration main effect, $F(2,14) = 25.64$, $P < 0.0001$, showed that rinse responses increased as a function of each ascending concentration. Analysis of the stimulus main effect, $F(2, 14) = 13.81$, $P < 0.001$, showed that average water-rinse responses following K_3Citrate containing stimuli, 0.17 ± 0.08, did not differ from average water-rinse responses following Na_3Citrate containing stimuli, 0.11 ± 0.05. However, average amiloride-rinse responses, 0.03 ± 0.01, following Na_3Citrate containing stimuli mixed with amiloride, were significantly smaller than either of the other average water-rinse responses ($P < 0.05$).

In summary, CT sucrose-response suppression for citric and hydrochloric acid increased as a function of titratable acidity, whereas acetic acid, the weakest acid, was slightly less effective. Trivalent citrate did not enhance sucrose responses, but suppressed baseline and sucrose evoked CT neural activity to a similar degree; these citrate suppressed CT responses rebounded with water rinses.

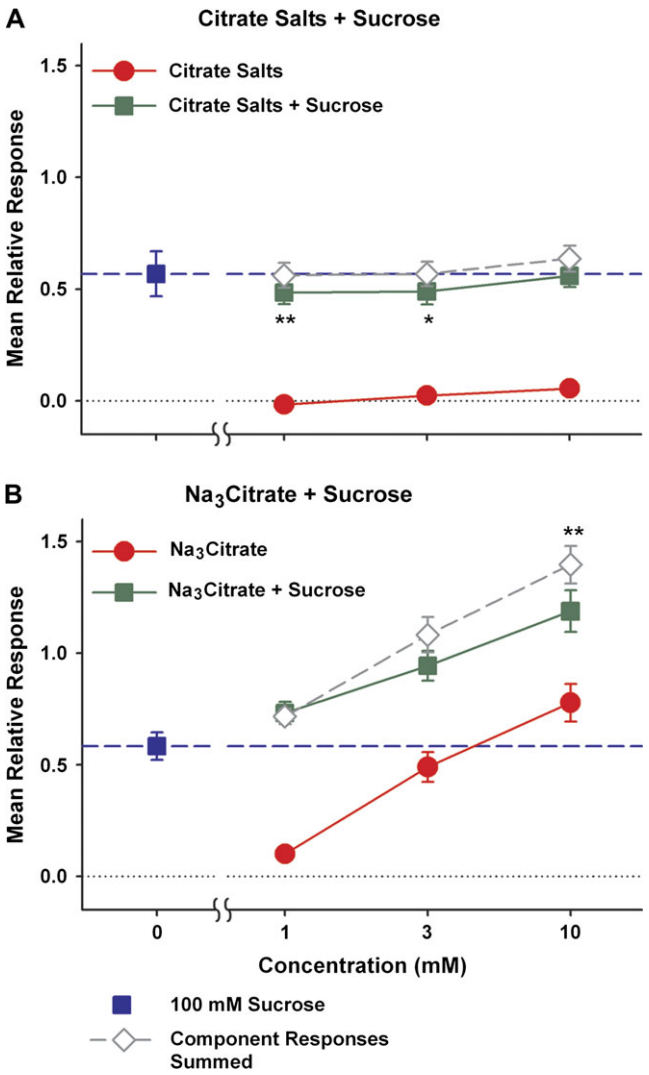

Figure 5 **(A)** Mean (±standard error of the mean [SEM]) CT responses to citrate salts plotted as a function of stimulus concentration. Citrate salts represent the combined mean of the responses to $Na_3Citrate$ mixed with amiloride and $K_3Citrate$. See text for an explanation. Average mixture responses with citrate salts at 1 and 3 mM were reliably smaller (*$P < 0.05$, **$P < 0.01$) than the average response to 100 mM sucrose alone and less than the component responses summed (open triangles) at every concentration ($P < 0.01$). **(B)** Mean (±SEM) CT responses to sucrose + $Na_3Citrate$ (without amiloride present) plotted as a function of stimulus concentration. Mixture responses were equivalent to the component responses summed at all concentrations except 10 mM, where the mixture response was still greater than either component presented alone but less than the component responses summed (**$P < 0.01$ mixture response vs. component responses summed).

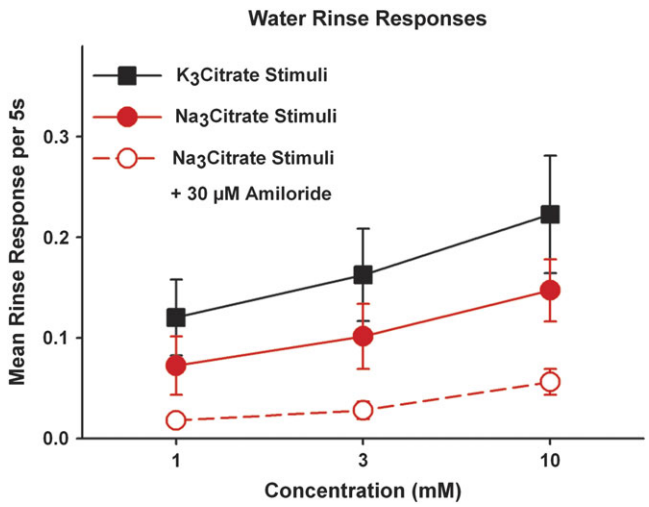

Figure 6 Mean (±standard error of the mean) rinse responses to citrate containing stimuli plotted as a function of stimulus concentration. Rinse responses to each sucrose + citrate salt mixture were averaged with the rinse responses to the citrate component alone. When averaged across the three concentrations, $K_3Citrate$ water rinses ≥ $Na_3Citrate$ water rinses > $Na_3Citrate$ amiloride rinses ($P < 0.05$).

Discussion

Summary of results

The current study demonstrates peripheral taste suppression in hamster whole-nerve CT responses to mixtures of sucrose with a mineral acid (HCl) and two carboxylic acids (acetic acid and citric acid). Previous work demonstrated that responses to HCl + sucrose mixtures were suppressed in sucrose-sensitive, but not acid-sensitive, CT single-fiber neurons (Hyman and Frank 1980b); thus, the suppression currently observed is considered an effect of acids on sucrose-sensing mechanisms. In the present study, sucrose-response suppression occurred at titratable acidity levels of 9–10 mEq/L for HCl and citric acid; but at 30 mEq/L for acetic acid, the weakest of the 3 acids. Dissociated proton concentrations did not predict sucrose-response suppression. Previous reports also show that stimulus pH does not predict psychophysical (Ganzevles and Kroeze 1987) or physiological (Beidler 1967, 1971; Ogiso et al. 2000) acid taste responses.

Responses to sucrose mixed with $K_3Citrate$ and $Na_3Citrate$ mixed with 30 µM amiloride were also suppressed. Citrate mixture suppression was compared directly with the average sucrose response for $K_3Citrate$ and $Na_3Citrate$ mixed with 30 µM amiloride. That is, mixture responses with $K_3Citrate$ and $Na_3Citrate$ mixed with 30 µM amiloride were smaller than the average response to 100 mM sucrose presented alone. In addition, baseline CT activity appeared reduced with $K_3Citrate$ application. Comparable overall mean water-rinse responses occurred after stimuli containing $K_3Citrate$ (0.17 ± 0.08) and $Na_3Citrate$ (0.11 ± 0.05).

The degree to which peripheral gustatory interactions occur may vary from species to species solely on the basis of peripheral anatomy (Miller 1971, 1974; Whitehead et al. 1999; Vandenbeuch et al. 2004; Zaidi and Whitehead 2006). The present hamster CT results apparently contrast with the previously reported enhanced number of action potentials elicited by citrate + sweetener mixtures in rat fungiform TRCs (Gilbertson et al. 1997). However, species

differences aside, there are considerable differences between recording responses from isolated TRCs in vitro and whole-nerve taste afferents. Isolated receptor cells cannot interact with other taste bud cells (Roper 2007) or send/receive possible divergent/convergent input from surrounding taste buds (Miller 1974; Whitehead et al. 1999), both mechanisms possible sources of peripheral taste modulation.

Substantial advances in the discovery of putative taste receptors has lead to the molecular identification of multiple T2Rs for bitter compounds (Adler et al. 2000; Chandrashekar et al. 2000), mGluR4 and the T1R1–T1R3 heterodimer for umami stimuli (Chaudhari et al. 1996; Nelson et al. 2002), T1R2–T1R3 for sweeteners (Nelson et al. 2001), acid-sensing ion channels (ASICs) (Ishimaru et al. 2006; Shimada et al. 2006; Huang et al. 2008), and an amiloride-sensitive ENaC for sodium transduction (Shigemura et al. 2008). Although molecular evidence for these receptors has yet to be demonstrated in hamsters, behavioral and functional similarities among hamsters, rats, and mice would argue for the existence of these receptors, in one form or another, across these species (Delay et al. 2008; Eschle et al. 2008). In the paragraphs that follow the impact of stimulus acidity on receptor function is explored with reference to putative taste receptors sequenced in other species.

Intercellular cross-talk

Various mechanisms have been proposed to account for acid taste transduction, from cytosolic acidification due to penetration of H^+ or undissociated acids (Lyall et al. 2001) to ASICs or transient receptor potential (TRP) channels that detect external acidity levels (Huang et al. 2006; Shimada et al. 2006). However, these mechanisms all involve depolarization of the TRC membrane and presumably ultimate afferent activation, not suppression.

Cross-talk between acid-sensing and sugar-sensing taste bud cells (Chandrashekar et al. 2006) may help explain the present results. Intercellular cross-talk between TRCs innervated by quinine-sensitive **E** neurons and sucrose-sensitive **S** neurons was considered previously as a possible mechanism for quinine inhibition of S-neuron sucrose responses (Frank et al. 2005). However, peripheral cross-talk between TRCs and the primary afferents that service sweet and sour stimulus modalities (Frank et al. 2005) are not quantitatively supported by our whole-nerve data. For example, in phase 2, equal response magnitudes to 10 mM citric acid (0.82 ± 0.06) and 10 mM HCl (0.80 ± 0.08) resulted in distinctly different levels of sucrose mixture suppression: 94 ± 7% for citric acid versus 40 ± 16% for HCl, $t(14) = 3.52$, $P < 0.01$. Although the distribution of responses to carboxylic acids among CT neuron types in hamsters is currently unknown, the mismatch between excitatory responses to acids and the inhibition of sucrose responses in the hamster CT suggests that acid transduction and acid suppression may have distinct sources.

Cytosolic acidification

It has long been known that acids penetrate cell membranes (Harvey 1914; Crozier 1916; Taylor 1928) and lower cytosolic pH (Gardner 1980; Spray et al. 1981). Application of acids to taste pores results in acidification of all cells in the taste bud (Richter et al. 2003), an event that accompanies acid taste transduction (Stewart et al. 1998; Lyall et al. 2001; Richter et al. 2003) as well as a myriad of other TRC membrane conductance changes (Spray et al. 1981; Moody 1984; Kinnamon et al. 1988; Zong et al. 2001; Stevens et al. 2001). There is evidence that internal acidification affects reactions taking place within the cytoplasm that could impact sensory transduction in individual receptor cells. For example, it has been shown that low cytosolic pH reduces stimulatory G_S protein activation of adenylate cyclase activity, thus reducing cyclic adenosine 3′,5′-monophosphate (cAMP) levels in NG108-15 cells (a mouse hybrid cell line) (Liu et al. 1999). In the gustatory system, reduced cAMP would likely decrease responses of taste cells responding to sugar and, thereby, decrease TRC output to the CT (Striem et al. 1989, 1991). In addition, recent evidence shows that intracellular acidification of Type II TRCs blocks neurotransmitter (adenosine triphosphate) release (Huang et al. 2008). Thus, transmission of gustatory information from Type II TRCs to the CT may be "short circuited" by intracellular acidification. This would predict that mixtures of acids with other stimuli known to have receptors on Type II TRCs, such as monosodium glutamate (MSG) or T2 receptor ligands, would also result in acid mixture suppression. Future whole-nerve and single-fiber CT experiments with acid + MSG mixtures would test this hypothesis.

External acidity level

Because TRC microvilli are first to come in contact with an acidic taste stimulus, before the acid penetrates the cell membrane, the suppression of acid + sucrose CT responses is likely the result of increased acidity in the immediate external lingual environment of the receptor itself. That is, when going from external acidity levels of 1 to 10 mEq/L, CT acid + sucrose mixture suppression is more likely related to changes in external acidity (e.g., pH optima of sugar receptors, allosteric H^+ binding) than increases in cytosolic acidity. Although external acidity is the adequate stimulus for TRC membrane-bound ASICs, ASICs or the TRP channel, PKD2L1 is located on a subset of TRCs distinct from those positive for the T1R2–T1R3 sugar receptor (Richter et al. 2003; Chandrashekar et al. 2006; Huang et al. 2006; Shimada et al. 2006). Thus, sucrose suppression is unlikely the result of direct increases in cytosolic acidity via ASICs on receptor cells positive for the T1R2–T1R3 heterodimer.

In addition, external acidity is known to have numerous effects on excitable membranes. Protons can alter the gating kinetics of Na^+ channels, titrate negative charges that attract cations to ion channel pores or alter acid groups within the channel pore itself (Hille 1992). Changes in extracellular

pH can also modulate ligand-gated ion channels, such as N-methyl-D-aspartate (Tang et al. 1990) and gamma-aminobutyric $acid_A$ receptors (Wilkins et al. 2002). Finally, inhibition of glycinergic spinal neurons, due to increases in extracellular acidity, was caused by a conformational change of the glycine receptor (GlyR) itself, not ASICs or cytoplasmic acidification (Li et al. 2003). Therefore, it is entirely possible that increases in sucrose mixture suppression, which accompany increases in external titratable acidity, reflect conformational changes in the binding sites of the putative T1R2–T1R3 sweetener receptor (Cui et al. 2006).

Responses to citrate and sucrose–citrate mixtures

K_3Citrate inhibited hamster CT neural spontaneous activity and induced a prolonged rebound water-rinse response regardless of the presence of sucrose (Figure 4), suggesting a "release" from citrate anion inhibition (Beidler 1967). CT water-rinse responses have also been observed after stimulating with HCl, sodium glutamate, and sodium benzoate in rat (Beidler 1967; Yamamoto and Kawamura 1974; DeSimone et al. 1995; Formaker et al. 2001). Anionic "water" responses, which are strikingly species dependent, were attributed to the balance between cationic and anionic binding sites in TRC plasma membranes (Beidler 1967) and thought to play a role in inhibitory interactions among taste papillae (Miller 1971, 1974).

Amiloride, which blocks ENaC within rodent TRC plasma membranes (Shigemura et al. 2005, 2008), specifically suppresses rodent CT responses to sodium and lithium salts. In the present study, we used 30 μM amiloride to eliminate 92% of the Na_3Citrate response. The remaining 8%, responses of electrolyte generalist neurons, were inhibited by low concentrations of citrate. The pattern of rebound rinse responses resembled CT neuronal responses when sodium salt anions were changed from acetate or glutamate to chloride (Rehnberg et al. 1993; Breza et al. 2008) and may suggest a possible role for Cl^- currents in electrolyte taste transduction (Formaker and Hill 1988; Herness and Sun 1999).

Baseline reductions to K_3Citrate and Na_3Citrate + amiloride were very similar, suggesting that amiloride-insensitive, electrolyte generalist neurons may have been inhibited. However, K_3Citrate water-rinse responses were larger than Na_3Citrate + amiloride-rinse responses (Figure 6), which also suggest that Na^+-specific CT neurons may contribute to the rebound rinse response. Further data on citrate salt responses of hamster CT single units are needed to determine which CT unit types are responsible for the observed effects.

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