Changes in taste receptor cell [Ca\(^{2+}\)]\(_i\) modulate chorda tympani responses to salty and sour taste stimuli

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Changes in taste receptor cell [Ca\(^{2+}\)]\(_i\), modulate chorda tympani responses to salty and sour taste stimuli. The relationship between taste receptor cell (TRC) Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) and rat chorda tympani (CT) nerve responses to salty [NaCl and NaCl + benzamil (Bz)] and sour (HCl, CO\(_2\), and acetic acid) taste stimuli was investigated before and after lingual application of ionomycin + Ca\(^{2+}\), 1,2-bis-(2-aminophenoxy)ethane-N,N',N''-tetraacetic acid acetoxymethyl ester (BAPTA-AM), U73122 (phospholipase C blocker), and thapsigargin (Ca\(^{2+}\)-ATPase inhibitor) under open-circuit or lingual voltage-clamp conditions. An increase in TRC [Ca\(^{2+}\)]\(_i\), attenuated the tonic Bz-sensitive NaCl CT response and the apical membrane Na\(^{+}\) conductance. A decrease in TRC [Ca\(^{2+}\)]\(_i\), enhanced the tonic Bz-sensitive and Bz-insensitive NaCl CT responses and apical membrane Na\(^{+}\) conductance but did not affect CT responses to KCl or NH\(_4\)Cl. An increase in TRC [Ca\(^{2+}\)]\(_i\), did not alter the phasic response but attenuated the tonic CT response to acidic stimuli. A decrease in [Ca\(^{2+}\)]\(_i\), did not alter the phasic response but attenuated the tonic CT response to acidic stimuli. In a subset of TRCs, a positive relationship between [H\(^+\)] and [Ca\(^{2+}\)]\(_i\) was obtained using in vitro imaging techniques. U73122 inhibited the tonic CT responses to NaCl, and thapsigargin inhibited the tonic CT responses to salty and sour stimuli. The results suggest that salty and sour taste qualities are transduced by [Ca\(^{2+}\)]\(_i\)-dependent and [Ca\(^{2+}\)]\(_i\)-independent mechanisms. Changes in TRC [Ca\(^{2+}\)]\(_i\), in a BAPTA-sensitive cytosolic compartment regulate ion channels and cotransporters involved in the salty and sour taste transduction mechanisms and in neural adaptation. Changes in TRC [Ca\(^{2+}\)]\(_i\), in a separate subcompartement, sensitive to inositol trisphosphate and thapsigargin but inaccessible to BAPTA, are associated with neurotransmitter release.

ionomycin; BAPTA; voltage clamp; U73122; benzamil

SEVERAL STUDIES SUGGEST THAT taste receptor cells (TRCs) respond with an increase in Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) when stimulated with sour (Lyall et al. 2003; Richter et al. 2003), sweet (Rebello and Medler 2010), bitter (Akabas et al. 1988; Medler 2010; Ogura et al. 2002; Rebello and Medler 2010), umami (Narukawa et al. 2006; Rebello and Medler 2010), and a mixture containing sweet and bitter taste stimuli (Huang and Roper 2010). Type III cells, termed presynaptic cells that express the polycystic kidney disease-like proteins PKD1L3 and PKD2L1, function as sour-sensing cells (Huang et al. 2006; Ishimaru et al. 2006; LopezJimenez et al. 2006) and possess specialized chemical synapses. Whereas PKD2L1 and PKD1L3 are reliable markers of sour-sensitive taste cells, PKD2L1 may have some role in sour transduction in fungiform taste cells, but neither PKD2L1 nor PKD1L3 plays a role in sour transduction in circumvallate taste cells (Horio et al. 2011). Weak organic acids enter across the apical membranes of TRCs as neutral molecules and decrease intracellular pH (pHi) (DeSimone and Lyall 2006). For strong acids, H\(^+\) entry is dependent on at least two proton-conductive pathways in the apical membranes of sour-sensing TRCs (Chang et al. 2010; DeSimone et al. 2011; Lyall et al. 2002a) that are amiloride and Ca\(^{2+}\) insensitive. One conductive pathway is activated by cAMP, and the second pathway depends on gp91phox, a component of the NADPH oxidase enzyme (DeSimone et al. 2011; Lyall et al. 2002a). Acidic stimuli depolarize type III cells and increase Ca\(^{2+}\) influx through voltage-gated Ca\(^{2+}\) channels (VGCCs) that are involved in the release of the neurotransmitters serotonin and norepinephrine from intracellular vesicles (Huang et al. 2008).

In fungiform TRCs, Na\(^{+}\) ions enter across the apical membrane by at least two pathways: one involving the apical amiloride- and Bz-sensitive epithelial Na\(^{+}\) channels (ENaCs) (Bosak et al. 2010; Chandrashekar et al. 2010; DeSimone et al. 1981) and the second involving putative transient receptor potential vanilloid (TRPV1) nonspecific cation channels (Lyall et al. 2004b). However, at present, in type I TRCs involved in Na\(^{+}\) sensing through ENaC (Dvoryanchikov et al. 2009; Vandenbeuch et al. 2008), the relationship between the increase in [Ca\(^{2+}\)]\(_i\) and neurotransmitter release is not clear. In addition to its role in the neurotransmitter release, alterations in TRC [Ca\(^{2+}\)]\(_i\), have been shown to modulate the chorda tympani (CT) taste nerve responses to salty (DeSimone and Lyall 2008; Lyall et al. 2009) and sour stimuli (Lyall et al. 2002a, 2004a, 2006). Although an increase in cytosolic [Ca\(^{2+}\)]\(_i\) seems to be critical for normal responses in TRCs (Medler 2010), at present, a detailed understanding of the relationship between TRC [Ca\(^{2+}\)]\(_i\), and neural responses to salty and sour taste stimuli is lacking.

The objective of this study was to investigate the effects of changes in TRC [Ca\(^{2+}\)]\(_i\), on rat CT responses to representative salty and sour taste stimuli. TRC [Ca\(^{2+}\)]\(_i\), was either increased or decreased by topical lingual application of a Ca\(^{2+}\)-ionophore, ionomycin, plus Ca\(^{2+}\) or a membrane-permeable Ca\(^{2+}\) chelator, 1,2-bis-(2-aminophenoxy)ethane-N,N,N',N''-tetraacetic acid acetoxyethyl ester (BAPTA-AM), respectively. The effects of BAPTA loading in TRCs were reversed by saturating the Ca\(^{2+}\)-binding sites on intracellular BAPTA by treating rat lingual TRCs in vivo with ionomycin+Ca\(^{2+}\). We also monitored changes in [Na\(^{+}\)]\(_i\), [Ca\(^{2+}\)]\(_i\), and [H\(^+\)]\(_i\) in TRCs using
imaging techniques in vitro. The data presented in this article suggest that changes in TRC \([Ca^{2+}]_i\) in two subcompartments, namely, an ionomycin- and BAPTA-sensitive cytosolic compartment and an intracellular inositol trisphosphate (IP3)- and thapsigargin-sensitive subcompartment, play an important role in regulating the neural responses to salty and sour taste stimuli. Changes in \([Ca^{2+}]_i\) in the cytosolic compartment regulate taste receptors/ion channels and cotransporters involved in the taste transduction mechanism and are involved in the neural adaptation to different taste qualities. Changes in TRC \([Ca^{2+}]_i\) in a separate subcompartment, inaccessible to \(Ca^{2+}\) buffering by BAPTA, are associated with neurotransmitter release and subsequent neural response.

**MATERIALS AND METHODS**

**In Vivo Studies**

**CT taste nerve recordings.** Animals were housed in the Virginia Commonwealth University (VCU) 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 VCU. Sixty female Sprague-Dawley rats (150–200 g) were used in this study. For CT recordings, rats were anesthetized by intraperitoneal injection of pentobarbital sodium (60 mg/kg), and supplemental pentobarbital sodium (20 mg/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° with a Deltaphase isotherm (model 39 DP; Braintree Scientific, Braintree, MA). The left CT nerve was exposed laterally as it exited the tympanic bulla and was placed onto a 32-gauge platinum-iridium wire electrode. An indifferent electrode was placed in nearby tissue. Neural responses to afferent stimuli were amplified by an isolation amplifier (ISO-80; World Precision Instruments, Sarasota, FL). Stimulus solutions were injected into a Lucite chamber (3 ml; 1 ml/s) affixed by vacuum to a 28-mm² patch of anterior dorsal lingual surface. The chamber was fitted with separate Ag-AgCl electrodes for passing of current and measuring potential. These electrodes served as inputs to a voltage- and current-clamp amplifier that permitted the recording of neural responses with the chemically stimulated receptive field under zero current clamp or voltage clamp. The clamp voltages were referenced to the mucosal side of the tongue (Ye et al. 1993, 1994). Integrated neural responses and lingual current and voltage changes were captured on disk using LabView software (National Instruments, Austin, TX) and analyzed off-line as described previously (Lyall et al. 2002a, 2004a, 2009). Student’s t-test was employed to analyze the differences between sets of data.

**Solutions.** The composition of the rinse and stimulating solutions is shown in Table 1. For the majority of the CT recordings, the rinse (R) and stimulating solutions were used without added CaCl₂. However, in those experiments in which we used ionomycin to load the cells with \(Ca^{2+}\), 1 or 10 mM CaCl₂ was added to the rinse and stimulating solutions.

TRCs were loaded in vivo with \(Ca^{2+}\) using 150 \(\mu\)M ionomycin (Sigma) in dimethyl sulfoxide (DMSO) for 45 min. In addition, rinse and stimulating solutions (Table 1) used for CT recordings following ionomycin treatment contained either 1 or 10 mM CaCl₂ (Lyall et al. 2002a, 2004a, 2009). TRCs were loaded in vivo with the \(Ca^{2+}\) chelator BAPTA-AM (Sigma) by dissolving BAPTA-AM directly in DMSO and applying it topically to the tongue for 45 min at a concentration of 13 or 33 mM as described previously (Lyall et al. 2009). The effects of BAPTA loading in TRCs were reversed by saturating the \(Ca^{2+}\)-binding sites on the intracellular BAPTA by treating the cells with ionomycin+\(Ca^{2+}\). To rat tongues already treated with BAPTA-AM, we topically applied 150 \(\mu\)M ionomycin to

**Table 1. Composition of solutions used in the CT experiments**

<table>
<thead>
<tr>
<th>Rinse</th>
<th>NaCl (10 mM KCl + 100 mM NaCl)</th>
<th>NaCl+bz (10 mM KCl + 100 mM NaCl + 0.005 mM Bz)</th>
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<tr>
<td>R (10 mM KCl)</td>
<td>R (10 mM KCl)</td>
<td>R (10 mM KCl)</td>
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<tr>
<td>R+Ca²⁺ (10 mM KCl + 10 mM CaCl₂)</td>
<td>R+Ca²⁺ (10 mM KCl + 10 mM CaCl₂)</td>
<td>R+Ca²⁺ (10 mM KCl + 10 mM CaCl₂)</td>
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<td>*R+Ca²⁺ (10 mM KCl + 10 mM CaCl₂)</td>
<td>*R+Ca²⁺ (10 mM KCl + 10 mM CaCl₂)</td>
<td>*R+Ca²⁺ (10 mM KCl + 10 mM CaCl₂)</td>
</tr>
<tr>
<td>R (10 mM KCl)</td>
<td>R (10 mM KCl)</td>
<td>R (10 mM KCl)</td>
</tr>
<tr>
<td>R+NaCl (100 mM NaCl)</td>
<td>R+NaCl (100 mM NaCl)</td>
<td>R+NaCl (100 mM NaCl)</td>
</tr>
<tr>
<td>R⁺AA (175 mM KCl + 100 mM HEPES, pH 6.1)</td>
<td>R⁺AA⁺Ca⁺ (175 mM KCl + 100 mM HEPES, pH 6.1)</td>
<td>R⁺AA⁺Ca⁺ (175 mM KCl + 100 mM HEPES, pH 6.1)</td>
</tr>
<tr>
<td>R⁺CO₂ (72 mM KCl + 100 mM HEPES, pH 7.4)</td>
<td>R⁺CO₂⁺Ca⁺ (72 mM KCl + 100 mM HEPES, pH 7.4)</td>
<td>R⁺CO₂⁺Ca⁺ (72 mM KCl + 100 mM HEPES, pH 7.4)</td>
</tr>
<tr>
<td>R (10 mM KCl)</td>
<td>R (10 mM KCl)</td>
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<td>R (10 mM KCl)</td>
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</table>

AA, acetic acid; Bz, benzamil; CPC, cetylpyridinium chloride. Rinse and stimulating solutions indicated with asterisks were used only in experiments with ionomycin.
the lingual surface for 30 min. Following ionomycin treatment, the lingual surface was perfused for 10 min with the rinse solution containing 10 mM KCl + 10 mM CaCl₂ and then perfused with 10 mM KCl rinse solution without CaCl₂ for another 10 min. This was done to remove external ionomycin and CaCl₂. For the CT recordings under control conditions, post-BAPTA-AM treatment and post-BAPTA-AM–post-ionomycin/100 mM NaCl treatment, the rinse and stimulating solutions used did not contain CaCl₂ (Table 1). CT responses were also recorded after topical lingual application of 250 μM U73122, a nonspecific blocker of phospholipase Cs (PLCs); its inactive analog, U73343 (Coleman et al. 2011); or 250 μM thapsigargin, a noncompetitive inhibitor of sarco(endo)plasmic reticulum Ca²⁺-ATPase (SERCA) (Rogers et al. 1995). All drugs were obtained from Sigma. Benzamil (Bz; 5 μM) and cetylpyridinium chloride (CPC; 2 mM) were added to the stimulating solutions to block Na⁺ entry into TRCs through ENaC and the putative TRPV1t, respectively (DeSimone et al. 2001; Lyall et al. 2004a). DMSO (Lyall et al. 1999), CaCl₂ (1 or 10 mM) (Lyall et al. 2002a, 2004a), or topical lingual application of 150 μM ionomycin by themselves (data not shown) did not alter the CT responses to taste stimuli.

In Vitro Studies

Studies with polarized fungiform taste bud preparations: Ca²⁺, Na⁺, and pH imaging. Rat lingual epithelium was isolated by collagenase treatment (Vinnikova et al. 2004). A small piece of the anterior lingual epithelium containing a single fungiform papilla was mounted in a special microscopy chamber as described previously (Lyall et al. 2001, 2002a). Relative changes in [Ca²⁺]i were monitored in polarized TRCs by loading the tissue with the Ca²⁺-sensitive fluoroprobe Fura-2 AM (10 μM; Molecular Probes) in the presence of 0.15% Pluronic at room temperature for 4 h. Before the experiment was started, the tissues were perfused on both sides with control solution for 15 min at the rate of 1 ml/min. TRCs were alternately excited at 340 and 380 nm and imaged at 15-s intervals. The emitted light was

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**Fig. 1.** Effect of ionomycin + Ca²⁺ on the chorda tympani (CT) response to NaCl. A: a representative CT response recorded while the tongue was superfused with 10 mM KCl + 10 mM CaCl₂ (rinse solution, *R+Ca²⁺*, see Table 1 for composition of rinse and stimulus solutions), 10 mM KCl + 10 mM CaCl₂ + 100 mM NaCl (NaCl+Ca²⁺), and 10 mM KCl + 10 mM CaCl₂ + 100 mM NaCl + 5 μM benzamil (Bz) (NaCl+Bz+Ca²⁺), and 10 mM KCl + 10 mM CaCl₂ + 100 mM NaCl + 5 μM Bz + 2 mM cetylpyridinium chloride (CPC) (NaCl+Bz+Ca²⁺+CPC). The CT response was recorded before (control) and after ionomycin + Ca²⁺ treatment (post-ionomycin). B: means ± SE of the normalized changes in the tonic CT response to NaCl+Ca²⁺, NaCl+Bz+Ca²⁺, and the Bz-sensitive component in 3 rats under control conditions and post-ionomycin. In each case, the NaCl CT responses were normalized to the corresponding CT responses obtained with 300 mM NH₄Cl. *P = 0.0001 (unpaired; n = 3). C: effect of ionomycin + Ca²⁺ on the voltage sensitivity of the tonic NaCl CT response. The NaCl CT responses were recorded at zero-current clamp and at −60 and +60 mV applied transepithelial voltage across the receptive field before (control) and after ionomycin + CaCl₂ treatment (post-ionomycin+Ca²⁺). In each case, the NaCl CT responses were normalized to the corresponding CT responses obtained with 300 mM NH₄Cl. Each point represents the mean ± SE of 3 animals. M ± SEM, means ± SE.
imaged at 510 nm. All experiments were done at room temperature (≈22°C).

Relative changes in [Na\(^{+}\)]\(_{i}\) were monitored in polarized TRCs by loading the tissue with a Na\(^{+}\)-sensitive fluoroprobe, Na-green. Before the experiment was started, the tissues were perfused on both sides with control Ringer solution for 15 min at the rate of 1 ml/min. TRCs were excited at 490 nm and imaged at 15-s intervals. The emitted light was imaged at 530 nm. All experiments were done at room temperature (≈22°C). Small regions of interest (ROIs) in the taste bud (diameter 2–3 μm) were chosen in which the changes in the fluorescence intensity at 490 nm (F\(_{490}\)) were analyzed using imaging software (TILLvision version 4.0.7.2; TILL Photonics, Martinsried, Germany). The background and autofluorescence at 490 nm were corrected from images of a taste bud without the dye. The F\(_{490}\) value under control conditions for each ROI was taken as 100%. Student’s t-test was employed to analyze the differences between sets of data (Lyall et al. 2002b).

In some experiments, taste bud cells were loaded with 2’,7’-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM) and changes in TRC pH\(_{i}\) were measured as described previously (Lyall et al. 2001, 2004a, 2006; Vinnikova et al. 2004).

Studies with isolated taste bud cells using confocal microscopy. Changes in [Ca\(^{2+}\)]\(_{i}\) were also monitored in isolated fungiform taste bud cells (Vinnikova et al. 2004) using confocal microscopy. The isolated taste buds were placed on a CellTak (Sigma)-coated coverslip that formed the bottom of a perfusion chamber and were perfused with Ringer solution (pH 7.4; Table 2). TRCs were loaded with fluo 3-AM (Molecular Probes). The taste buds were incubated at room temperature with Ringer solution containing 30 μM of the dye for 1 h. After this, the cells were washed with control solution. The measurements were made using a Zeiss LSM 410 confocal microscope. The cells were excited by the 488-nm spectral line of the argon-ion laser, and the images were taken at the emission wavelength of 530 ± 10 nm (E\(_{530}\)). In TRCs loaded with fluo-3, the changes in E\(_{530}\) were expressed relative to the fluorescence emission intensity of different ROIs under control conditions. The E\(_{530}\) value under control conditions for each ROI was taken as 100% and was compared between different ROIs under different experimental conditions. Student’s t-test was employed to analyze the differences between sets of data.

Solutions. The composition of the Ringer solutions used in the imaging experiments is shown in Table 2. At the physiological pH and osmolarity, the relationship between changes in pH\(_{i}\) and [Ca\(^{2+}\)]\(_{i}\) was studied.
investigated by briefly exposing the isolated fungiform TRCs or the basolateral membrane of polarized fungiform TRCs to Ringer solutions containing 30 mM Na-acetate, 15 mM NH₄Cl or unilaterally decreasing the basolateral Na⁺ concentration from 150 mM to 0 as described previously (Lyall et al. 2004a, 2006; Vinnikova et al. 2004).

RESULTS

Effect of Changes in TRC [Ca²⁺] on the CT Responses to Mineral Salts

Studies with ionomycin + Ca²⁺. The CT response to 100 mM NaCl + 10 mM CaCl₂ (Table 1, NaCl+Ca²⁺) exhibited a Bz-sensitive and a Bz-insensitive component (Fig. 1A, control). The Bz-insensitive component (NaCl+Bz+Ca²⁺) was inhibited to the rinse baseline level by perfusing the tongue with NaCl+Bz+Ca²⁺ + 2 mM CPC (DeSimone et al. 2001; Lyall et al. 2004b). Following ionomycin treatment, both the phasic (transient) and tonic (steady state) CT responses to NaCl+Ca²⁺ were smaller relative to control. However, the tonic CT responses to NaCl+Bz+Ca²⁺ and NaCl+Bz+Ca²⁺+CPC were not different from control (Fig. 1A, post-ionomycin). Following treatment with ionomycin + 10 mM CaCl₂, the decrease in tonic NaCl CT response was due to a specific decrease in the Bz-sensitive component ([NaCl+Ca²⁺] – (NaCl+Bz+Ca²⁺)) of the NaCl CT response (Fig. 1B; P = 0.0001, unpaired; n = 3). The Bz-insensitive but CPC-sensitive tonic NaCl CT response was not affected. In contrast, when the rinse and NaCl solutions contained 1 mM CaCl₂, ionomycin treatment had no effect on the CT response to NaCl+Ca²⁺ or NaCl+Bz+Ca²⁺ (Table 1) (data not shown). Presumably, with only 1 mM extracellular Ca²⁺ in the rinse and stimulating solutions, the increase in TRC [Ca²⁺] in the presence of ionomycin is insufficient to inhibit the Bz-sensitive NaCl CT response.

Relative to the response at zero-current clamp (0 mV), the magnitude of the tonic NaCl+Ca²⁺ CT response increased at 60 mV and decreased at +60 mV (Fig. 1C; control). Treatment with ionomycin + 10 mM CaCl₂ significantly attenuated both the magnitude of the tonic NaCl+Ca²⁺ CT response at 0 mV and the voltage sensitivity of the response (slope of the CT response with change in voltage) (Fig. 1C, post-ionomycin). Following...
ionomycin treatment, the slope of the response (2.7 ± 0.2 \times 10^{-3} \text{ response units/mV}) was smaller relative to its value (4.8 ± 0.3 \times 10^{-3} \text{ response units/mV}) under control conditions ($P = 0.009$, unpaired; $n = 3$). The observation that the size of the response and the slope of the response (i.e., the voltage sensitivity or response conductance) are proportional suggests that a decrease in the Bz-sensitive NaCl CT response is due to a decrease in the apical membrane Na$^+$ conductance. No significant effect was observed on the CT responses to KCl after ionomycin ($10 \mu$M) treatment, the slope of the response (4.7 ± 0.04 \times 10^{-3} \text{ response units/mV}) was greater relative to its value (3.4 ± 0.07 \times 10^{-3} \text{ response units/mV}) under control conditions ($P = 0.0001$, unpaired; $n = 3$). These results suggest that the observed increase in the TRC [Ca$^{2+}$], specifically inhibits the Bz-sensitive NaCl CT response.

*Studies with BAPTA-AM.* Chelating TRC [Ca$^{2+}$], with 33 mM BAPTA-AM enhanced both the phasic and tonic NaCl CT responses relative to control (Fig. 2A, NaCl). Most of the increase in the tonic NaCl CT response in the presence of BAPTA was due to an increase in the Bz-sensitive [NaCl – (NaCl+Bz)] component of the NaCl CT response. Treating the tongue with 33 mM BAPTA-AM enhanced the tonic Bz-sensitive part of the NaCl CT response by 44 ± 2\% relative to control (Fig. 2B; $P = 0.005$, unpaired; $n = 3$). A small but significant increase in the tonic Bz-insensitive component of the NaCl CT response was also observed after BAPTA treatment ($P = 0.024$).

BAPTA-AM treatment significantly enhanced the magnitude of the tonic NaCl CT response at 0 mV and the voltage sensitivity of the CT response (Fig. 2C, post-BAPTA) relative to control (Fig. 2C, control). Following BAPTA treatment, the slope of the response (4.7 ± 0.04 \times 10^{-3} \text{ response units/mV}) was greater relative to its value (3.4 ± 0.07 \times 10^{-3} \text{ response units/mV}) under control conditions ($P = 0.0001$, unpaired; $n = 3$). These results suggest that the observed increase in the Bz-sensitive and Bz-insensitive NaCl CT responses is due to an increase in the TRC apical membrane Na$^+$ conductance.

*Studies with post-BAPTA–post-ionomycin+Ca$^{2+}$ treatment.* BAPTA-induced enhancement of the phasic and tonic components of the NaCl CT response was reversed by saturating the Ca$^{2+}$-binding sites on the intracellularly trapped BAPTA with ionomycin + 10 mM CaCl$_2$ (Fig. 3, A–C). It is important to note that among several salty and sour taste stimuli tested, BAPTA (13 mM) specifically enhanced the CT response to NaCl. BAPTA treatment produced no significant effect on the phasic and tonic components of the CT response to 100 mM Na$^+$-free solution containing 150 mM NaCl and 1 mM CaCl$_2$ (pH 7.4).

**Fig. 4.** Regulation of unilateral apical Na$^+$ influx by taste receptor cell (TRC) Ca$^{2+}$ concentration ([Ca$^{2+}$]). A: the transmitted image of a polarized fungiform taste bud mounted in our custom perfusion chamber and viewed with a ×40 objective from the basolateral side. B: the fluorescence image excited at 490 nm after the cells were loaded with Na-green. The image in A shows that the dye is specifically loaded in the TRCs within the taste bud (arrow). Bar, 50 μm. C: a polarized fungiform taste bud preparation loaded with Na-green was initially perfused on both sides with Na$^+$-free solution containing 150 mM N-methyl-D-glucamine-Cl (pH 7.4; 1 mM CaCl$_2$). Temporal changes in fluorescence intensity at 490 nm ($F_{490}$) were monitored while the apical compartment was perfused with the control Ringer solution containing 150 mM NaCl (pH 7.4; 1 mM CaCl$_2$). In the second part of the experiment, the apical and basolateral membranes were perfused with 0 Na$^+$–0 Ca$^{2+}$ solution for 15 min. Following this, the temporal changes in $F_{490}$ were monitored while the apical membrane was perfused with 150 mM Na$^+$–0 Ca$^{2+}$ Ringer solution (pH 7.4; 0 Ca$^{2+}$). In each region of interest (ROI), the $F_{490}$ value in 0-Na$^+$ Ringer solution was normalized to 100%. The changes in $F_{490}$ are presented as %change in fluorescence relative to 0 Na$^+$, and values are means ± SE of $n$ where $n$ = no. of ROIs within the taste bud. D: summary of the data from 3 individual polarized fungiform taste bud preparations. *$P = 0.0073$ (unpaired)."
KCl or 300 mM NH₄Cl (Fig. 3, D and E). These results suggest that K⁺ and NH₄⁺ conductive pathways in the apical membrane of TRCs are not affected by a decrease in TRC [Ca²⁺].

Effect of Changes in [Ca²⁺] on the Unilateral Apical Na⁺ Flux in Polarized Fungiform TRCs

In polarized fungiform taste buds loaded with Na-green (Fig. 4, A and B), the unilateral Na⁺ flux was measured as a temporal increase in F₄₉₀ in response to an increase in apical Na⁺ concentration from 0 to 150 mM (Fig. 4C) (Lyall et al. 2002b). The unilateral apical Na⁺ flux was measured when the polarized fungiform taste bud preparations were perfused bilaterally with Ringer solutions containing 1 mM Ca²⁺ or 0 Ca²⁺ (pH 7.4; Table 2) (Fig. 4C). The mean unilateral apical Na⁺ flux was enhanced significantly (P = 0.0073, unpaired; n = 3; 23 ROIs) in Ca²⁺-free Ringer solution (Fig. 4D). When the polarized taste buds were reperfused with control Ringer solution (pH 7.4), the unilateral apical Na⁺ flux decreased to its control level (Fig. 4D). The changes in unilateral apical Na⁺ influx observed in our in vitro experiments are most likely related to the ionomycin- and BAPTA-induced effects on the NaCl CT responses in vivo shown in Figs. 1, 2, and 3, A–C.

Effect of Changes in TRC [Ca²⁺] on the CT Responses to Sour Stimuli

Studies with ionomycin +Ca²⁺. We have previously shown that tonic CT responses to HCl, CO₂, or acetic acid solutions containing 10 mM CaCl₂ are decreased after the tongue is treated with 150 μM ionomycin (Lyall et al. 2003, 2004a).

Studies with BAPTA-AM. Loading TRCs in vivo with 13 mM BAPTA-AM inhibited the tonic CT response to 10, 20, and 30 mM HCl without affecting the phasic component of the HCl CT response (Fig. 5A). The mean phasic CT response to 20 mM HCl was not affected by BAPTA-AM treatment relative to control (Fig. 5B) (Lyall et al. 2003, 2004a). BAPTA-AM at 13 mM (Fig. 6, A and B) and 33 mM (Fig. 6, C and D) induced a concentration-dependent decrease in the tonic CT response to CO₂, acetic acid, and HCl (raw data not shown) without affecting the phasic component of the CT response. The mean phasic CT responses to CO₂ and acetic acid were not affected by BAPTA-AM treatment relative to control (data not shown). BAPTA induced a dose-dependent decrease in the tonic CT response to acidic stimuli (Figs. 7, A–C). In each case, titrating the Ca²⁺-binding sites on the intracellular BAPTA with ionomycin +Ca²⁺ restored the CT response to acidic stimuli close to their control values. Taken together, the results with ionomycin +Ca²⁺ and BAPTA-AM treatment indicate that the transduction mechanism for both strong and weak organic acids involves an increase in TRC [Ca²⁺].

Relationship Between pHᵢ and [Ca²⁺]ᵢ in Isolated Fungiform Taste Buds

Figure 8A shows a pseudocolor confocal fluorescence emission image of an isolated taste bud loaded with fluo 3 and perfused with control Ringer solution (Table 2; pH 7.4). Figure 8A shows that the fluorescent dye is taken up by all TRCs within the plane of the imaged taste bud. Figure 8B shows the same plane after 1 min following the replacement of the control Ringer solution with a Ringer solution containing 30 mM Na-acetate in the perfusion chamber (Table 2; pH 7.4). The changes in fluo 3 fluorescence are related to the pHᵢ changes observed in a separate set of isolated fungiform taste bud cells (Fig. 8E) loaded with BCECF and exposed to a basolateral
Na-acetate pulse as in Fig. 8D. Figure 8C shows that individual TRCs and specific regions within a single TRC respond with changes in fluorescence intensity differently. In 16 ROIs investigated within the taste bud, 8 ROIs demonstrated an increase in fluorescence intensity (i.e., an increase in [Ca\(^{2+}\)]) during the acidification phase of the Na-acetate pulse (Fig. 8C, a–b). Although not shown, 5 ROIs responded with a decrease in fluorescence (i.e., a decrease in [Ca\(^{2+}\)]), and 3 ROIs demonstrated no significant change in fluorescence. In another isolated taste bud, 12 of 18 ROIs demonstrated an increase in fluo 3 emission fluorescence (i.e., an increase in [Ca\(^{2+}\)]) during the acidification phase of the basolateral Na-acetate pulse (Fig. 8D, a–b) and a partial decrease in fluorescence during the spontaneous recovery from acidic pH (Fig. 8D, b–c). Upon Na-acetate washout, the alkalization phase of the Na-acetate pulse was accompanied by a decrease in fluo 3 emission fluorescence (i.e., a decrease in [Ca\(^{2+}\)]) (Fig. 8D, c–d), and the spontaneous recovery phase from the alkaline pH\(_i\) (Fig. 8D, d–e) was accompanied by an increase in fluo 3 emission fluorescence close to its baseline value.

The relationship between pH\(_i\) and [Ca\(^{2+}\)] was also investigated in intact polarized fungiform taste bud preparations by briefly exposing the basolateral membrane of TRCs to Ringer solution containing 15 mM NH\(_4\)Cl or 0 Na\(^+\) (Vinnikova et al. 2004). The results presented in Fig. 9A show that during the NH\(_4\)Cl pulse, the initial increase in TRC pH\(_i\) (Fig. 9B, a–b) is accompanied by a decrease in [Ca\(^{2+}\)] (a decrease in fura 2 fluorescence intensity ratio (FIR)), and upon NH\(_4\)Cl washout, a decrease in pH\(_i\) (Fig. 9B, c–d) is accompanied by an increase in [Ca\(^{2+}\)] (increase in fura 2 FIR). A similar relationship between pH\(_i\) and [Ca\(^{2+}\)] was observed in four individual polarized fungiform taste bud preparations exposed to brief basolateral NH\(_4\)Cl pulses (a total of 26 ROIs within the 4 taste buds). In a separate polarized fungiform taste bud preparation, [Ca\(^{2+}\)] increased (Fig. 9C, a–b) as TRC pH\(_i\) acidified during the basolateral Na\(^+\) removal (Fig. 9D, a–b). Upon reperfusion...
of the basolateral membrane with 150 mM NaCl, the pH$_i$ recovered to its resting value (Fig. 9D, c–d), accompanied by a decrease in [Ca$^{2+}$]$_i$. A similar relationship between pH$_i$ and [Ca$^{2+}$], was observed in three individual polarized fungiform taste bud preparations exposed to basolateral 0 Na$^+$ Ringer solution (a total of 18 ROIs within the 3 taste buds). These results suggest that within a taste bud TRCs are heterogeneous with respect to their responses to acid stimulation. Only those cells which demonstrate a strict inverse relationship between pH$_i$ and [Ca$^{2+}$]$_i$ are likely to be involved in sour taste transduction.

**Effect of U73122 on the CT Responses to Salty Stimuli**

U73122 inhibited the tonic CT responses to 100 mM NaCl (Fig. 10A). In the presence of U73122, the decrease in NaCl CT response was due to a decrease in both the Bz-sensitive and Bz-insensitive components of the NaCl CT response (Fig. 10, A and B). U73122 did not inhibit the control CT response to 0.3 M NH$_4$Cl (Fig. 10B). U73343, its inactive analog, had no effect on CT responses to any taste stimuli tested (Coleman et al. 2011; Lyall et al. 2010). These data suggest that CT responses to salty stimuli are also regulated by one or more PLCs in salt-sensing TRCs.

**Effect of Thapsigargin on the CT Responses to Salty and Sour Stimuli**

Topical lingual application of thapsigargin inhibited the tonic CT responses to NH$_4$Cl, NaCl, and HCl relative to control (Fig. 11). These data suggest that CT responses to salty and sour stimuli are also partially dependent on Ca$^{2+}$ release from internal stores.

**DISCUSSION**

The main effects of changes in TRC cytosolic [Ca$^{2+}$], on the tonic CT responses to salty and sour taste stimuli along with the proposed taste receptor/ion channels, transporters, and intracellular signaling intermediates regulated by [Ca$^{2+}$], are summarized in Table 3. Overall, the data suggest that, similar to the sweet, bitter, and umami taste qualities (DeSimone et al. 2012), salty and sour taste qualities are also transduced by [Ca$^{2+}$]-dependent and [Ca$^{2+}$]-independent mechanisms.

**Changes in Cytosolic [Ca$^{2+}$], Regulate ENaC and the Bz-Sensitive NaCl CT Response**

In inside-out patch recordings of M1 cells (mouse kidney cortical collecting duct cells) elevating [Ca$^{2+}$], inhibited ENaC open probability without altering the channel conductance. The inhibitory effect was due to a direct interaction between Ca$^{2+}$ and ENaC, and was dependent on [Ca$^{2+}$]$_i$ (Gu 2008). Here, we show that changes in TRC [Ca$^{2+}$], regulate 1) unilateral apical Na$^+$ influx (Fig. 4); 2) apical membrane Na$^+$ conductance (Figs. 1C and 2C); and 3) the magnitude of the Bz-sensitive NaCl CT response (Fig. 1, A and B; Fig. 2, A and B; and Fig. 3, A–C). Decreasing TRC [Ca$^{2+}$], with BAPTA specifically enhanced the CT response to NaCl (Fig. 3, A–C). Taken together, these results suggest that salt sensing involves changes in TRC Ca$^{2+}$ in at least two separate subcompartments. TRCs have a cytosolic [Ca$^{2+}$], compartment in which [Ca$^{2+}$], involved in taste transduction may be buffered with BAPTA and is associated with regulating ENaC activity. In a separate subcompartment that does not seem to be accessible to BAPTA, [Ca$^{2+}$], is mobilized during neurotransmitter release.

In a subset of TRCs, a decrease in pH$_i$ is accompanied by an increase in [Ca$^{2+}$], and an increase in pH$_i$ with a decrease in [Ca$^{2+}$], (Figs. 8 and 9) (Lyall et al. 2003). We have previously shown that a decrease in TRC pH$_i$ decreases ENaC activity and inhibits the magnitude of the Bz-insensitive NaCl CT response, and an increase in pH$_i$ enhances ENaC activity and increases the Bz-insensitive NaCl CT response (Lyall et al. 2002b). This suggests that changes in TRC pH$_i$ can directly and/or indirectly regulate ENaC activity via secondary changes in [Ca$^{2+}$], (Gu 2008). It is expected that an increase in both H$^+$, (i.e., a decrease in pH$_i$) and [Ca$^{2+}$], will have an additive effect in inhibiting ENaC activity and thus in decreasing the Bz-sensitive NaCl CT response. Similarly, an increase in pH$_i$ and the accompanying decrease in [Ca$^{2+}$], could have an additive effect in enhancing the Bz-sensitive NaCl CT response. Thus
[Ca$^{2+}$]$_i$ and [H$^+$]$_i$ are important regulators of ENaC in salt-sensing TRCs and of the Bz-sensitive NaCl CT response. The regulation of ENaC activity by pH varies in different tissues and in different species. In *Xenopus* oocytes expressing human α-, β-, and γ-ENaC subunits, amiloride-sensitive current was enhanced at pH 6.0 and decreased at pH 8.5 relative to pH 7.4. In contrast, the pH-induced changes were not observed with rat α-, β-, and γ-ENaC subunits expressed in oocytes (Collier and Snyder 2009a). This difference in pH sensitivity was attributed to species differences in the γ-ENaC subunit. An additional ENaC subunit, the δ-subunit, is activated by acidic pH (Ji and Benos 2004). However, the δ-ENaC subunit is not expressed in the rat or in human renal epithelia (Collier and Snyder 2009a). Since this subunit is expressed in a subset of human TRCs (Huque et al. 2009), it raises the possibility that the regulation of ENaC in human TRCs by pH may differ from the rat responses reported in this study.

It is suggested that changes in pH alter human ENaC activity by modulating Na$^+$ self-inhibition (Collier and Snyder 2009b). In rats, TRC ENaC activity and the Bz-sensitive NaCl CT response are also regulated by Na$^+$ self-inhibition (De-Simone and Lyall 2008; Gilbertson and Zhang 1998). However, the modulation of NaCl CT responses by pH$_i$ and [Ca$^{2+}$]$_i$.
under the experimental conditions described in this study cannot be explained by alterations in Na⁺ self-inhibition of the ENaC activity in TRCs. Changes in H⁺ and [Ca²⁺]ᵢ most likely inhibit the Bz-insensitive NaCl CT responses in rats by directly inhibiting the ENaC activity in TRCs (Fig. 4) (Gu 2008; Lyall et al. 2002b).

Changes in Cytosolic [Ca²⁺], Regulate TRPV1t and the Bz-Insensitive NaCl CT Responses

The Bz-insensitive NaCl CT response is enhanced by decreasing TRC [Ca²⁺], (Fig. 2B). We have previously shown that the TRPV1t-dependent Bz-insensitive NaCl CT response is 1) modulated by vanilloids, CPC, ethanol, nicotine, Maillard reacted peptides, and elevated temperature; 2) inhibited by capsazepine and SB-366791; and 3) absent in TRPV1 knockout mice (Lyall et al. 2004b). Changes in TRC [Ca²⁺] modulate the agonist concentration versus the magnitude of the Bz-insensitive NaCl CT response relationships by altering channel activity indirectly through protein kinase C- and calcineurin-dependent changes in the phosphorylation-dephosphorylation state of the channel protein (Lyall et al. 2009).

About 40% of the CT response to KCl and NH₄Cl can be accounted for by the influx of K⁺ and NH₄⁺ via the putative apical TRPV1t cation channel (Lyall et al. 2004b). Since BAPTA treatment only produced a small change in the Bz-insensitive NaCl CT response (Fig. 2B), it is expected that changes in TRC [Ca²⁺] would also have only minimal effect on the K⁺ and NH₄⁺ flux through the putative TRPV1t cation channel (Fig. 3, D and E). Thus an increase in TRC [Ca²⁺] is not a prerequisite requirement for eliciting a CT response to KCl or NH₄Cl. The CT responses to KCl are also insensitive to changes in [Ca²⁺] and pH (Lyall et al. 2002b).

In TRCs loaded with 33 mM BAPTA-AM, it is expected that the cytosolic [Ca²⁺] is buffered at a value much below normal. However, under the conditions where the cytosolic [Ca²⁺] is close to zero, we observed a bigger NaCl CT response relative to control (Fig. 2, A and B, and Fig. 3, A–C). If an increase in the cytosolic [Ca²⁺] is a prerequisite for the release of the neurotransmitter (Finger et al. 2005; Huang and Roper 2010), one would predict that in the presence of intracellular BAPTA, no CT response would have been observed. This suggests that changes in cytosolic [Ca²⁺] are mostly involved in the regulation of ENaC and TRPV1t activity and are not associated with the neurotransmitter release from TRCs onto CT nerve fibers. The latter compartment associated with the neurotransmitter release does not seem to be affected by ionomycin or BAPTA treatment under the in vivo and in vitro experimental conditions used in this study.

Changes in Cytosolic [Ca²⁺], Regulate CT Responses to Acidic Stimuli

In polarized TRCs, stimulating the apical membrane with acidic stimuli elicited a decrease in TRC pH (Lyall et al. 2001). At constant external pH (pHₒ), inducing a rapid decrease in pH with the use of short basolateral pulses of Na-acetate or NH₄Cl induced an increase in [Ca²⁺], in a subset of TRCs (Figs. 8 and 9). Conversely, intracellular alkalinization was accompanied by a decrease in [Ca²⁺], (Lyall et al. 2003). This positive relationship between [H⁺] and [Ca²⁺] is important for acid taste transduction for both strong and weak organic acids (Richter et al. 2003). In many cases, weak organic acids are good stimuli even when presented at near-neutral pH. In this case, weak organic acids can permeate the apical membrane and enter TRCs as neutral undisassociated molecules. Once inside the cell, the undisassociated molecules dissociate to yield H⁺ ions (Lyall et al. 2001). Facilitating apical H⁺ entry by incorporating the K⁺/H⁺ exchanger nigericin in the apical membrane of TRCs in vivo elicits a CT
response at pHs close to the neutral pH (Sturz et al. 2011). Thus the proximate signal for both strong and weak acid transduction is a decrease in TRC pHi, and a downstream signaling event is the pHi-induced increase in [Ca\(^{2+}\)]\(_i\) (DeSimone and Lyall 2006).

At present, the exact mechanism as to how a change in pHi induces an increase in [Ca\(^{2+}\)]\(_i\) is not clear. Since in mouse TRCs removing extracellular Ca\(^{2+}\) reduced acid-evoked Ca\(^{2+}\) responses but depleting intracellular Ca\(^{2+}\) stores with thapsigargin had no effect, it was suggested that acid taste responses are generated by an influx of extracellular Ca\(^{2+}\). Based on the observations that acid-evoked Ca\(^{2+}\) responses could be blocked by Ba\(^{2+}\) and Cd\(^{2+}\), the data further suggested that Ca\(^{2+}\) influx occurred through the VGCCs (Richter et al. 2003).

BAPTA treatment produced a differential effect on the phasic and tonic components of the CT responses to acidic stimuli (Figs. 5 and 6). This suggests that the initial transient phasic CT response is independent of changes in [Ca\(^{2+}\)]\(_i\) in the cytosolic compartment but depends on Ca\(^{2+}\) increase in a subcompartment in TRCs that is not accessible by BAPTA. The transduction mechanisms for phasic and tonic components of the CT responses to acidic stimuli are different. The phasic component of the CT response depends on the pHi-induced cell shrinkage and the activation of a basolateral flufenamic acid-
vesicle fusion responsible for transmitter release is triggered by

IP3 and Thapsigargin-Dependent \[Ca^{2+}\], Subcompartment

\[
\text{IP}_3^- \text{ and Thapsigargin-Dependent } \text{[Ca}^{2+}\text{], Subcompartment}
\]

An increase in \([Ca^{2+}]\), is required for the release of neurotransmitter from the presynaptic terminals in all neurons (Llinas and Moreno 1998). An increase in \([Ca^{2+}]\), in the microdomains against the cytoplasmic surface of the plasma-membrane during transmitter release suggests that the synaptic vesicle fusion responsible for transmitter release is triggered by the activation of a low-affinity \(Ca^{2+}\)-binding site at the active zone (Llinas and Moreno 1998). However, the relationship between changes in \([Ca^{2+}]\), in the cytosolic compartment and the synaptic regions of the TRCs is not known at present. In our studies, depleting intracellular \(Ca^{2+}\) stores with thapsigargin reduced CT responses to salt and sour taste stimuli (Fig. 11). Ryanodine receptors contribute to some taste-evoked signals that are dependent on \(Ca^{2+}\) release from internal stores. In a subset of type III cells, blocking ryanodine receptors reduced an increase in \(Ca^{2+}\) induced by depolarizing the membrane potential with high \(K^+\) (Rebello and Medler 2010). This suggests that ryanodine receptors may be functionally coupled to VGCCs in a subset of type III cells and may contribute to the depolarization-induced \(Ca^{2+}\) signal.

The decrease in the Bz-insensitive NaCl CT response in the presence of U73122 is most likely due to changes in PIP2 and its interactions with TRPV1t (Lyall et al. 2010). Besides PLC\(\beta_2\), several other PLC isoforms (PLC\(\beta_1\), PLC\(\beta_2\), PLC\(\beta_3\), and PLC\(\gamma_1\)) have been shown to be present in fungiform and circumvallate TRCs (Chandrashekar et al. 2006; Hacker et al. 2008; Lyall et al. 2010; Toyono et al. 2005). U73122 affects the Bz-insensitive NaCl CT response by inhibiting one or more PLC isoforms other than the PLC\(\beta_2\) isoform and increasing membrane PIP2 levels. The Bz-sensitive NaCl CT response decreased in the presence of U73122 (Fig. 10, A and B). In A6 cells, an increase in PIP2 increased ENaC activity by direct interaction with \(\beta\)- or \(\gamma\)-ENaC subunits (Yue et al. 2002), and inhibiting PLC with U73122 enhanced ENaC activity by increasing the mean open time of the channel (Pochynyuk et al. 2008a). These results suggest that PIP2 regulation of ENaC may be tissue and cell specific. In addition to PIP2, a physiological role for phosphatidylinositol-3-OH kinase (PI3-K) and its product, phosphatidylinositol 3,4,5-trisphosphate (PIP3), in modulating ENaC activity has been documented (Pochynyuk et al. 2008b). It is likely that U73122-induced changes in membrane PIP2 levels affect ENaC activity indirectly via changes in PIP3.

Thus, although a decrease in BAPTA-sensitive cytosolic \([Ca^{2+}]\), enhances the Bz-sensitive NaCl CT response, inhibiting \(IP_3^-\) generation (Fig. 10, A and B) or depleting intracellular \(Ca^{2+}\) stores with thapsigargin (Fig. 11) attenuated the CT response. Thapsigargin also attenuated CT responses to HCl relative to control (Fig. 11). These results tend to suggest that even in salt-sensing and sour-sensing TRCs, neurotransmitter release may also be partially dependent on a \(IP_3^-\) and thapsigargin-sensitive \([Ca^{2+}]\), subcompartment.

In summary, changes in pH, and cytosolic \([Ca^{2+}]\), modulate Na+ influx through apical ENaCs and hence modulate the Bz-sensitive NaCl CT response. In contrast, Na+ transport through the putative TRPV1t nonspecific cation channel is much less sensitive to changes in cytosolic \([Ca^{2+}]\) and \([H^+]\), (Lyall et al. 2002b). For acidic stimuli, a decrease in TRC pH, (Lyall et al. 2004a) elicits an increase in cytosolic \(Ca^{2+}\), and activation of basolateral NHE-1, which leads to a decrease in the tonic CT response to acid stimulation, and hence causes adaptation of the neural response. The phasic part of the CT response to acidic stimuli is independent of cytosolic \(Ca^{2+}\) but must involve an increase in \([Ca^{2+}]\), in a subcompartment. Since HCl CT responses are enhanced by cAMP (DeSimone et al. 2011; Lyall et al. 2002a), there may be cAMP and \(Ca^{2+}\) signaling to integrate tastant-evoked signals in a subset of type III cells.

### Table 3. Regulation of Salty and Sour CT Responses by \([Ca^{2+}]\)

<table>
<thead>
<tr>
<th>Taste Stimuli</th>
<th>Response Measured</th>
<th>Ionomycin+(Ca^{2+}) (↑ TRC ([Ca^{2+}]))</th>
<th>BAPTA-AM (↑ TRC ([Ca^{2+}]))</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl, NaCl+Bz</td>
<td>Bz-sensitive NaCl CT response (NaCl -- (NaCl+Bz))</td>
<td>↓</td>
<td>↑</td>
<td>ENaC</td>
</tr>
<tr>
<td>NaCl, NaCl+Bz</td>
<td>Bz-insensitive NaCl CT response (NaCl+Bz)</td>
<td>↑, ↑</td>
<td>↑</td>
<td>TRPV1t</td>
</tr>
<tr>
<td>NaCl+Bz, NaCl+Bz+RTX*</td>
<td>RTX concentration vs. magnitude of Bz-insensitive NaCl CT response</td>
<td>↓</td>
<td>↑</td>
<td>TRPV1t</td>
</tr>
<tr>
<td>NaCl</td>
<td>Apical membrane Na+ conductance</td>
<td>↓</td>
<td>↓</td>
<td>ENaC</td>
</tr>
<tr>
<td>KCl</td>
<td>KCl CT response</td>
<td>↓</td>
<td>↑</td>
<td>ENaC</td>
</tr>
<tr>
<td>NH4Cl</td>
<td>NH4Cl CT response</td>
<td>↑</td>
<td>↑</td>
<td>TRPV1t</td>
</tr>
<tr>
<td>HCl, CO2, acetic acid</td>
<td>HCl, CO2, acetic acid CT response</td>
<td>↓</td>
<td>↓</td>
<td>NHE-1</td>
</tr>
<tr>
<td>NaCl</td>
<td>Unilateral apical Na+ influx</td>
<td>↓</td>
<td>↑</td>
<td>ENaC</td>
</tr>
</tbody>
</table>

Arrows indicate effects (↓, decrease; ↑, increase; or +, no change) of ionomycin+\(Ca^{2+}\) (increased taste receptor cell \([Ca^{2+}]\)) and BAPTA-AM (decreased taste receptor cell \([Ca^{2+}]\)) in chorda tympani (CT), Na+ conductance, or Na+ influx response to various taste stimuli. ENaC, epithelial Na+ channels; RTX, resiniferatoxin. *Data are from Lyall et al. (2010). The phasic CT responses to acidic stimuli are \(Ca^{2+}\) independent. †The increase in adaptation occurs due to \([Ca^{2+}]\)-induced activation of the basolateral Na+/H+ exchanger 1 (NHE-1) (Lyall et al. 2002a, 2004a). ‡About 30–40% of the CT response to KCl and NH4Cl can be accounted for by the flux of \(K^+\) and NH4+ through the putative TRPV1t (variant of transient receptor potential vanilloid-1) cation channels (DeSimone et al. 2001).
Sensory response

Effect of [Ca^{2+}]

Changes in cell volume

Modulation of rat chorda tympani NaCl responses and intracellular Na^+ activity in polarized taste receptor cells by pH.

The receptors and cells for mammalian taste.

A novel pharmacological probe links the amiloride-insensitive NaCl, KCl, and NH_4Cl chorda tympani taste responses.

Voltage-gated H^+ channels in the chorda tympani nerve responses to strong acids.

Delocalization of rat taste receptor cell [Ca^{2+}] responses to bitter, sweet, and umami taste stimuli.

Modulation of taste receptor cell [Ca^{2+}], modulate chorda tympani responses to bitter, sweet, and umami taste stimuli.

Inward rectifier channel, ROMK, is localized to the apical tips of glia-like cells in mouse taste buds.


Involvement of NADPH-dependent and cAMP-PKA-sensitive H^+ channels in the chorda tympani nerve responses to strong acids.


