Self-Inhibition in Ca\textsuperscript{2+}-Evoked Taste Responses: A Novel Tool for Functional Dissection of Salt Taste Transduction Mechanisms

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Kloub, Mamoun A., Gerard L. Heck, and John A. DeSimone. Self-inhibition in Ca\textsuperscript{2+}-evoked taste receptors: a novel tool for functional dissection of salt taste transduction mechanisms. J. Neurophysiol. 79: 911–921, 1998. Rat chorda tympani (CT) responses to CaCl\textsubscript{2} were obtained during simultaneous current and voltage clamping of the lingual receptive field. Unlike most other salts, CaCl\textsubscript{2} induced negatively directed transepithelial potentials and gave CT responses that were auto-inhibitory beyond a critical concentration. CT responses increased in a dose-dependent manner to \(0.3\) M, thereafter they decreased with increasing concentration. At concentrations where Ca\textsuperscript{2+} was self-inhibitory, it also inhibited responses to NaCl, KCl, and NH\textsubscript{4}Cl present in mixtures with CaCl\textsubscript{2}. Ca\textsuperscript{2+} completely blocked the amiloride-insensitive component of the NaCl CT response, the entire KCl-evoked CT response, and the high-concentration-domain CT responses of NH\textsubscript{4}Cl (\(\approx0.3\) M).

The overlapping Ca\textsuperscript{2+}-sensitivity between the responses of the three Cl\textsuperscript{-} salts (Na\textsuperscript{+}, K\textsuperscript{+}, and NH\textsubscript{4}\textsuperscript{+}) suggests a common, Ca\textsuperscript{2+}-sensitive, transduction pathway. Extracellular Ca\textsuperscript{2+} has been shown to modulate the paracellular pathways in different epithelial cell lines by decreasing the water permeability and cation conductance of tight junctions. Ca\textsuperscript{2+}-induced modulation of tight junctions is associated with Ca\textsuperscript{2+} binding to fixed negative sites. This results in a conversion of ion selectivity from cationic to anionic, which we also observed in our system through simultaneous monitoring of the transepithelial potential during CT recording. The data indicate the paracellular pathway as the stimulatory and modulatory site of CaCl\textsubscript{2} taste responses. In addition, they indicate that important transduction sites for NaCl, KCl, and NH\textsubscript{4}Cl taste reception are accessible only through the paracellular pathways. More generally, they show that modulation of paracellular transport by Ca\textsuperscript{2+} in an intact epithelium has functional consequences at a systemic level.

INTRODUCTION

Taste buds and ion-transporting epithelia share common topology, including polarized receptor cells with apical and basolateral domains separated by tight junctions (TJs) (Holland et al. 1989). The early events in the detection of Na\textsuperscript{+} salts in the rat peripheral taste system also share similarities with other ion-transporting epithelia. The Na\textsuperscript{+}-detecting elements include an amiloride and voltage-sensitive transduction site (Avenet and Lindemann 1988; DeSimone et al. 1981; Heck et al. 1984; Schifffman et al. 1983; Ye et al. 1993). This suggests that one transducer is an apical membrane ion channel, similar to that involved in epithelial sodium transport (Smith and Benos 1991). However, amiloride, does not suppress the entire chorda tympani (CT) response to NaCl in rats, even at high concentration (Brand et al. 1985; DeSimone and Ferrell 1985; Elliot and Simon 1990; Formaker and Hill 1988; Lundy and Contreras 1997). Evidence suggests that the amiloride-insensitive component (AIC) of the NaCl neural response arises from transduction sites along the basolateral membranes of receptor cells, access to which is assumed to be through paracellular pathways (Elliot and Simon 1990; Mierson et al. 1996; Simon et al. 1993; Stewart et al. 1995; Ye et al. 1993). The principal barriers in these pathways are the TJs that connect the apical poles of the taste receptor cells, which act as weakly cation-selective barriers (DeSimone et al. 1984; Simon and Garvin 1985; Ye et al. 1993). In addition, these pathways likely participate in K\textsuperscript{+} and NH\textsubscript{4}\textsuperscript{+} salt taste responses (Kloub et al. 1997; Ye et al. 1994). If so, transduction sites for NaCl, KCl, and NH\textsubscript{4}Cl may be accessible only by means of a common pathway across the TJ complex. However, until now, a reliable means of probing paracellular pathways, involved in taste reception, has been unavailable.

Calcium modulation of TJs has been documented in various epithelia. Removing it increases the permeability of the rat intestine (Tidball 1964), opens the junctional complex between the oxyntic cells (Sedar and Forte 1964) and pancreatic acinar cells (Meldolesi et al. 1978), and produces fragmentation of the TJs in mammary glands (Pitelka et al. 1983). Removal of Ca\textsuperscript{2+} from the medium of Madin-Darby canine monolayer cells opens their TJs, and its subsequent restoration causes them to reseal (Martinez-Palmo et al. 1980). Ca\textsuperscript{2+} triggers the sealing of TJs at a critical concentration by acting on an extracellular site (Contreras et al. 1991). TJ permeability has been correlated with changes in transepithelial conductance (TEC) and extracellular Ca\textsuperscript{2+} concentration is inversely proportional to TEC (Contreras et al. 1991). These observations suggest that Ca\textsuperscript{2+} can be used to probe the common paracellular pathways believed to be involved in part of the CT response to NaCl and NH\textsubscript{4}Cl and virtually all of that to KCl. There are suggestions that Ca\textsuperscript{2+} effects on TJs can modulate other intact epithelia (Mooseker 1985), but this has not yet been demonstrated. Our results from an intact taste sensory system verify this speculation.

At concentrations where Ca\textsuperscript{2+} is self-inhibitory, it also inhibits responses to NaCl, KCl, and NH\textsubscript{4}Cl in mixtures with CaCl\textsubscript{2}. Moreover, transepithelial potential (TEP) recordings, made simultaneously with the CT responses, confirm that Ca\textsuperscript{2+} converts the paracellular region from cation- to anion-selective, consistent with observations on TEP in vitro in other epithelia (Moreno and Diamond 1974; Prather and Wright 1969; Smyth and Wright 1966). The data and their interpretation using electrodiffusion theory show that Ca\textsuperscript{2+} blocks transduction sites for Na\textsuperscript{+}, K\textsuperscript{+}, and NH\textsubscript{4}\textsuperscript{+} taste recep-
tion by reducing the permeability of the paracellular pathway to cations in a highly cooperative fashion.

METHOIDS

Solutions and chemicals

Stimulus salts included NaCl, NH₄Cl (Mallinkrodt Chemical, Paris, KY) KCl, and CaCl₂ and amiloride hydrochloride (Sigma Chemical, St. Louis, MO). All chemicals were reagent grade and were prepared in distilled water. A rinse consisting of 15 mM KHCO₃ + 15 mM KCl (pH 8.3) was applied for 1 min before and after each test stimulus. NaCl-depleted Krebs-Henseleit buffer was applied periodically to the tongue as an artificial saliva (cf. Ye et al. 1994).

Nerve preparation and voltage-clamp recording

Neural responses were obtained from the CT nerves of female Sprague-Dawley rats (180–240 g) during chemical stimulation of the tongue, according to the protocols approved by the Virginia Commonwealth University Animal Research Committee. The surgical procedure has been described in detail (Ye et al. 1993, 1994). Rats were preanesthetized with ether and then given an intraperitoneal injection of pentobarbital sodium (65 mg/kg) with additional injections as needed. The trachea was cannulated and the left chorda tympani nerve was exposed, cut, and placed on a platinum electrode. Petroleum jelly was placed around the CT, and a platinum reference electrode was positioned nearby. A stimulation chamber was held on the anterior tongue by vacuum. Solutions were injected in 3-mI aliquots at 1 ml/s and remained in the chamber for 1 or 2 min. The whole CT neural activity was analyzed and displayed as described previously (Ye et al. 1993). Transepithelial voltage or current clamp was maintained with a four-electrode voltage clamp amplifier as described elsewhere (Ye et al. 1993). A periodic (15 s) biphasic pulse of 1 μA (current clamp) or 20 mV (voltage clamp) was generated to measure the transepithelial resistance.

Data analysis

Integrated CT responses were analyzed off-line as previously described (Ye et al. 1993). The area under an integrated CT response curve for 1 min from the onset of neural activity was used as its numerical value. All stimulus series were bracketed by application of 0.1 M NaCl, and their CT responses were included only when bracketing NaCl responses varied by <20%. All responses for a given animal were normalized to those of 0.1 M NaCl. The normalized TEC for CaCl₂ was expressed as the conductance per unit ionic strength. Phasic CT responses were not analyzed separately because of stimulus flow rate sensitivity (Heck and Erickson 1973; Smith and Bealer 1975). Numerical results are expressed as the means ± SE. Statistical significance was determined by paired Student’s t-test.

RESULTS

CaCl₂ transepithelial potential and CT responses

Figure 1 (top) shows TEP changes while recording the CT responses to CaCl₂ at zero current clamp. The TEP evoked by NaCl showed an electropositive increase on the submucosal side because paracellular regions normally behave as leaky cation-exchangers (Ye et al. 1993, 1994). In contrast, the TEPs evoked by CaCl₂ increased in the electronegative direction, evidence of a reversal in ion exchange selectivity in favor of anions (cf. Fig. 2C). Figure 1 also shows CT recordings to NaCl before and after a series of responses to three concentrations of CaCl₂ under zero current clamp. CaCl₂ CT responses are unusual in displaying strong self-inhibition: <0.3 M, CT responses to CaCl₂ increase in a dose-dependent manner; however, >0.3 M responses decline significantly. This is established further by the complete concentration-response relations, under zero current clamp, shown in Fig. 2A. Recording the CT concentration-response relation for CaCl₂ with the lingual receptive field under voltage clamp (Fig. 2A) preserves the fundamental shape of the curve but shifts it to the right on the concentration axis at ΔV = +50 mV and to the left at ΔV = −50 mV. This suggests that the effect of voltage is mainly to increase (negative clamp voltage) or decrease (positive clamp voltage) the Ca²⁺ concentration at sites in the main permeability barriers. Monitoring changes in TEC along with the CT response provides some insight into the origin of the unusual CT response changes. Figure 2B shows a plot of the normalized conductance as a function of CaCl₂ concentration. As concentration increased, the normalized CaCl₂ TECs decreased. Between 0.1 and 0.3 M CaCl₂ there was a rapid drop in the TECs, whereafter (0.3 M) a smaller drop was observed. These data demonstrate that Ca²⁺ binding causes a reduction in paracellular CaCl₂ conductance. (see Analysis of self-inhibition in Ca²⁺-evoked taste responses and discussion).

Effect of Ca²⁺ on NaCl CT responses and TEPs

Figure 3 shows the effect of CaCl₂ on the CT response to 0.3 M NaCl. The NaCl CT response displayed the usual electronegative-going TEP. In mixture with 0.1 M CaCl₂, the TEP for NaCl was electronegative but clearly reduced, indicating diminishing relative cation conductance, but the CT response was not significantly reduced. However, given that 0.1 M CaCl₂ alone presents a CT response (cf. Fig. 1),

![Transepithelial potential response](image-url)
a significant suppression in overall CT response, therefore, occurred. With 0.1 M CaCl₂, the NaCl TEC showed no further increase even though the ionic strength had doubled, so the conductance per unit ionic strength declined by 50%. Similar to CaCl₂ CT responses, the NaCl + CaCl₂ mixture suppression then was correlated with a large drop in TEC. In mixture with 0.2 M CaCl₂ and higher concentrations, the TEP for NaCl became electronegative, the TEC continued to drop but more slowly, and the corresponding CT responses showed obvious suppression. Figure 4 illustrates CaCl₂-induced CT response suppression for both 0.1 and 0.3 M NaCl. In each case, suppression became pronounced beyond 0.2 M CaCl₂. At 0.3 M CaCl₂, the response to 0.1 M NaCl was suppressed by 8% and that to 0.3 M NaCl by 47%, figures approximating the AICs of the responses to 0.1 and 0.3 M NaCl, respectively (Ye et al. 1993). Nearly a constant difference between the mixture responses and that due to CaCl₂ remained at all CaCl₂ concentrations >0.3 M. If CaCl₂
0.3 M CaCl$_2$ and then a mixture of CaCl$_2$ with NaCl. The inhibition by amiloride. This is followed by a response to CaCl$_2$ at this, and higher concentrations, is often at or all next record demonstrates that the AIC of NaCl CT response is entirely Ca$^{2+}$ blocked access to paracellular transduction sites (presumed locus of the AIC for NaCl), the difference remaining ought to be suppressed entirely by amiloride at all CaCl$_2$ concentrations $>0.3$ M because it must arise exclusively from apical Na-channel transduction sites.

Figure 5 displays records testing the preceding hypothesis. Figure 5, top, shows a response to 0.3 M NaCl and its partial inhibition by amiloride. This is followed by a response to 0.3 M CaCl$_2$ and then a mixture of CaCl$_2$ with NaCl. The next record demonstrates that all of the NaCl response that occurs in a mixture with CaCl$_2$ is amiloride-sensitive, i.e., CaCl$_2$ blocked the AIC of the NaCl response, as suggested in Figs. 3 and 4. This effect is even more striking in mixtures with 0.5 M CaCl$_2$ (Fig. 5, bottom) because the CT response to CaCl$_2$ at this, and higher concentrations, is often at or near the baseline. As trace N + C$_2$ + A shows again, all of the AIC was eliminated and the response to NaCl was driven to baseline by the presence of both Ca$^{2+}$ and amiloride. These results demonstrate that the AIC of NaCl CT responses is entirely Ca$^{2+}$ sensitive.

**Effect of Ca$^{2+}$ on KCl CT responses**

Figure 6 shows the effect of using a series of CaCl$_2$ concentrations in mixture with 0.3 M KCl. Complete suppression of KCl CT responses by CaCl$_2$ did not occur until the CaCl$_2$ concentration in the mixture was $\sim$0.3 M. When the CaCl$_2$ concentration in the mixture exceeded 0.3 M, KCl responses were, in fact, not significantly different from responses observed with CaCl$_2$ alone (Fig. 6, inset). Ca$^{2+}$ affects K$^+$ responses in a manner similar to the AIC of NaCl response (cf. Figs. 4 and 5). This would be expected if transduction sites for both Na$^+$ and K$^+$ are accessible only by way of a paracellular pathway, blockable by Ca$^{2+}$.

**Effect of Ca$^{2+}$ on NH$_4$Cl CT responses**

Figure 7A shows recordings from experiments demonstrating the effect of two concentrations of CaCl$_2$ in mixture with either 0.1 or 0.3 M NH$_4$Cl on the CT responses to NH$_4$Cl. The chosen NH$_4$Cl concentrations are representative of the low (0.1 M) and high (0.3 M) concentration regimes observed in CT responses to NH$_4$Cl (Kloub et al. 1997). Figure 7A (top) shows a response to 0.3 M NH$_4$Cl (1st record) followed by responses to mixtures of 0.3 M NH$_4$Cl and 0.3 M CaCl$_2$ (2nd record) and 0.3 M NH$_4$Cl and 0.5 M CaCl$_2$ (3rd record). The suppression of 0.3 M NH$_4$Cl response by both concentrations of CaCl$_2$ is obvious. In contrast, Fig. 7A (bottom) shows that the responses to mixtures of 0.1 M NH$_4$Cl and either concentration of CaCl$_2$ significantly exceed the response to 0.1 M NH$_4$Cl alone. An examination of the concentration-response relation for the two concentrations of NH$_4$Cl over a range of CaCl$_2$ (Fig. 7B) shows that the responses to 0.1 M NH$_4$Cl and CaCl$_2$ are nearly additive over the range of CaCl$_2$ concentration. On the other hand, responses to 0.3 M NH$_4$Cl and CaCl$_2$ follow the KCl pattern, i.e., a much less-than-additive response $<0.3$ M CaCl$_2$ and a response not significantly different from that of CaCl$_2$ alone at CaCl$_2$ concentrations $>0.3$ M.
Next, response to 0.3 M CaCl$_2$ followed by a repeat stimulation with 0.3 M NaCl / CaCl$_2$ mixture contains amiloride. Latter represents the amiloride-insensitive component (AIC) of NaCl CT response. On achieving steady state response, 0.3 M CaCl$_2$ was replaced by the mixture of NaCl and CaCl$_2$, each at 0.3 M. Additional response caused by NaCl in mixture with CaCl$_2$ represents the amiloride-sensitive component (ASC) of NaCl as is demonstrated in the next trace where the NaCl/CaCl$_2$ mixture contains amiloride. Note the elimination of the NaCl-evoked response. Bottom: response to 0.3 M NaCl followed by the response to 0.3 M NaCl + 0.5 M CaCl$_2$, the latter represents the ASC of NaCl CT response. This is evident from the next trace where the application of amiloride completely eliminated the entire response.

M. Similar Ca$^{2+}$ effects on NH$_4$Cl CT responses were observed when presented in mixtures with 0.5 M NH$_4$Cl (data not shown). The major qualitative differences between 0.1 and 0.3 M NH$_4$Cl in mixtures with CaCl$_2$ once again, emphasize that NH$_4$Cl CT responses are the result of two transduction mechanisms. One of these predominates at concentrations approximately >0.3 M and is believed to involve NH$_4$Cl transport across paracellular pathway (Kloub et al. 1997). The fact that the high NH$_4$Cl concentration CT response regime is more sensitive to Ca$^{2+}$ is further confirmation of this view.

Analysis of self-inhibition in Ca$^{2+}$-evoked taste responses

Figures 1 and 2 suggest that the self-inhibition in the CaCl$_2$ response results from CaCl$_2$ having to diffuse across a paracellular barrier to reach the Ca$^{2+}$ sensors on the basolateral membranes of taste-bud cells. The reversal in the TEP (Fig. 2C) suggests that in passing through the diffusion barrier Ca$^{2+}$ causes a reduction in Ca$^{2+}$ ion permeability relative to that of Cl$^-$. From the rapid decline in conductance observed between 0.1 and 0.3 M CaCl$_2$ (Fig. 2B), this appears to occur through a highly cooperative reaction between Ca$^{2+}$ and fixed anionic sites. With a minimum of further assumptions, therefore, the nonmonotonic CT response-concentration curves at zero current and under voltage clamp, the CaCl$_2$ conductance-concentration curve and the electro-negative shift in TEP with increasing CaCl$_2$ concentration should emerge from a straightforward application of the electrodiffusion equations. The analysis will help illustrate that the permeability barrier attenuates large disturbances in ion concentration, osmotic pressure, and pH that may arise in the oral cavity. The extracellular fluid on the submucosal side of the barrier is only slightly perturbed, consequently, cell volume changes will be negligible.

Zero-current clamp

The CaCl$_2$ flux across the diffusion barrier between the mucosal side (oral cavity) and the submucosal side is

$$J = -P_{c2} \Delta c$$

where $c$ is the CaCl$_2$ concentration difference between the submucosal (s) side, $c_s$, and the mucosal (m) side, $c_m$, and

$$P_{c2} = \frac{3P_1P_2}{2P_1 + P_2}$$

Here $P_1$ and $P_2$ are the permeability coefficients of Ca$^{2+}$ and Cl$^-$, respectively. The corresponding potential difference under zero current is

$$\Delta \psi = \frac{RT}{F} \ln \frac{c_s}{c_m}$$

where $\Delta \psi$ is potential between sides s and m and $RT/F$ has its usual meaning. For convenience, the dimensionless potential, $\phi_0$ (-F$\Delta \psi$/RT) also will be used.

We restrict the analysis to the pseudo-steady state (tonic
FIG. 7. A: integrated CT responses under 0 current clamp for 2 concentrations of NH₄Cl (0.1 and 0.3 M) alone or in a mixture with 2 concentrations of CaCl₂ (0.3 and 0.5 M). Top: response to 0.3 M NH₄Cl followed by the responses of the mixtures, 0.3 M NH₄Cl/0.3 M CaCl₂ (C₁) and 0.3 M NH₄Cl/0.5 M CaCl₂ (C₂). Note that NH₄Cl CT responses were suppressed in the presence of CaCl₂. Bottom: response to 0.1 M NH₄Cl followed by the responses of the mixtures, 0.1 M NH₄Cl/0.3 M CaCl₂ (C₁) and 0.1 M NH₄Cl/0.5 M CaCl₂ (C₂). Note that the responses to mixtures of 0.1 M NH₄Cl and either concentration of CaCl₂ significantly exceed the response to 0.1 M NH₄Cl alone. B: mean normalized CT responses as a function of CaCl₂ concentration in mixture with 0.3 M NH₄Cl (・) or 0.1 M NH₄Cl (○) or without NH₄Cl (△) under 0 current clamp. Each point represents the mean ± SE (n = 5). Note that CaCl₂ exhibits differential effects on the responses to each concentration of NH₄Cl. Responses of the mixture of 0.1 M NH₄Cl and CaCl₂ and those to CaCl₂ alone are nearly additive, but nearly a complete suppression of 0.3 M NH₄Cl responses occurred in mixtures with CaCl₂ when the CaCl₂ concentration in the mixture was ≈ 0.3 M.

phase) of the CT response. This state is assumed to be sustained by a first order process that removes Ca²⁺ ions from the submucosal compartment at the same rate that they enter by diffusion from the mucosal side. This would be the case if, for example, the inflowing CaCl₂ can diffuse into the extracellular fluid space. The steady state condition is

\[ J = \frac{aV}{A} c_s \]  

where \( J \) is the Ca²⁺ influx into the s compartment (cf. Eq. 4), \( a \) is rate constant, \( V \) is the volume, and \( A \) is the area of the s compartment. Substituting for \( J \) in Eq. 4 using Eq. 1 gives the s concentration, \( c_s \), as a function of the m concentration, \( c_m \), viz

\[ c_s = \frac{c_m}{1 + \frac{P_{10}}{P_{22}}} \]  

where \( \beta = \frac{aV}{A} \). Ca²⁺ self-inhibition of Ca²⁺ permeability can be modeled as

\[ P_1 = \frac{P_{10}}{1 + \left( \frac{c_m}{k} \right)^n} \]  

where \( P_{10} \) is the Ca²⁺-permeability coefficient at low (0) mucosal CaCl₂ concentration, \( k \) is the Ca²⁺ binding dissociation constant, and \( n \) is a number greater than one that expresses the cooperative aspect of the decrease in Ca²⁺ permeability (cf. Fig. 2B). Introducing the relative permeability, \( p (P_{10}/P_2) \), Eq. 5 becomes

\[ c_{at} = \frac{c_m}{1 + \frac{2}{3} \left( 1 + \frac{c_m}{k} \right)^2 \left( \frac{1}{p} \right)} \]  

where the dimensionless constant \( \gamma = \beta/P_2 \) is an index of diffusion control in the transfer and elimination of the stimulus from the s compartment, and \( c_{at} \) is the zero current value of \( c_s \). The larger the \( \gamma \) value, the smaller will be the \( c_{at} \) for a given value of \( c_m \). It is apparent that the paracellular barrier substantially attenuates the stimulus intensity before it reaches the sensory cells, i.e., the barrier shrinks the variation of the stimulus submucosal concentration (see further).

Voltage clamp

Under voltage-clamp the Ca²⁺ influx, \( J_1 \), is given by

\[ J_1 = -P_i \Delta c \left[ 1 + \frac{2 \phi}{\ln (c_i/c_m)} \right] \]  

where \( \phi \) is the dimensionless potential under voltage clamp. Imposing the steady state condition (Eq. 4) gives
\begin{equation}
2\phi = -\left[1 + \left(\frac{2}{P}\right)\left(\frac{x}{x+1}\right)\right] \ln x \tag{9}
\end{equation}

where \( P = P_1/P_2 \) and \( x = c_n/c_{m} \). Equation 9 is transcendental in \( x \), precluding a closed form expression for \( c_n \). In addition, the dissociation constant, \( k \), connected with \( P \), can be expected to be potential dependent, further complicating solution. An alternative is to seek solutions for small voltage perturbations about zero current. The dimensionless voltage \( \phi \) represents deviations from \( \phi_0 \) viz.

\begin{equation}
\phi = \phi_0 + v \tag{10}
\end{equation}

where \( v \) is the dimensionless perturbation voltage. If \( v \) is restricted to values less than one, i.e., to \( \sim 26 \text{ mV} \), a solution has the form

\begin{equation}
x = x_0 e^{-2\nu} \tag{11}
\end{equation}

where \( x_0 \) is \( c_n/c_{m} \) under zero current conditions, and \( \delta \) is the voltage modulator function, which depends on a quantity \( q \), given by

\begin{equation}
q = \frac{2(P_1 - P_2)}{2P_1 + P_2} \tag{12}
\end{equation}

which is twice the permeability factor that determines open-circuit potential (cf. Eq. 3). The voltage modulator function, \( \delta \), is 1/\( h(q) \) where \( h(q) \) is

\begin{equation}
h(q) = q + \left(\frac{q - 1}{2}q + \frac{q + 2}{2}\right) \ln \left[\frac{q + 2}{q + 2 + 2\gamma}\right] \tag{13}
\end{equation}

The significance of the voltage modulator function, \( \delta \), is detailed in the following text.

From Eq. 11 the submucosal Ca\(^{2+}\) concentration, \( c_n \), under voltage clamp is given by \( c_{n0} \exp[-2\nu] \), and with Eq. 7 this is

\begin{equation}
c_n = \frac{c_{m0} e^{-2\nu}}{1 + \frac{2}{3} \left[2 + \left(\frac{c_{m0} e^{-2\nu}/k)^n}{\gamma}\right)\right]} \tag{14}
\end{equation}

where the voltage dependent factor in \( k \) (i.e., \( \exp[2\nu] \)) also has been included. The predicted values of \( c_n \) as a function of \( c_{m0} \) are displayed in Fig. 8 (top) for the three voltage conditions: \( v = -1 \), \( v = 0 \), and \( v = +1 \), and for \( \gamma = 150 \) (Fig. 8, curves A–C, respectively; see legend for the values of the other parameters). The extent of diffusion control in the system, as expressed in the value of \( \gamma \), is unknown a priori. However, because \( \gamma \) determines the maximum concentration of Ca\(^{2+}\) achieved on the submucosal side of the diffusion barrier, it is possible to put bounds on it. Table 1 shows the maximum value of \( c_n \) at zero current as a function of the corresponding index of diffusion control, \( \gamma \) for the choice of parameters used in Fig. 8. To minimize the chances of cytotoxicity, it seems likely that the degree of diffusion control will be such to prevent the maximum value of \( c_n \) from rising no more than a few millimolar above its basal level. This would tend to restrict \( \gamma \) to values greater than \( \sim 25 \) but with an upper limit of \( \sim 150 \). The profiles of \( c_n \) as a function of \( c_{m0} \) in Fig. 8, accurately reflect the CT response-concentration curves for CaCl\(_2\) in Fig. 2A. This suggests that taste cell responses are simply proportional to the changes in Ca\(^{2+}\) that occur in the submucosal microenvironment.

**Voltage modulator function**

Figure 8 (bottom) shows \( \delta \) as a function of \( c_{m0} \) for the same parameter values used in Fig. 8 (top), \( \delta \) has a maximum value of 0.3 at \( c_{m0} = 0 \), so diffusing Ca\(^{2+}\) ions “feel” at maximum only 30% of the clamp voltage at low Ca\(^{2+}\) concentration where the diffusion barriers are still cation selective. As they become increasingly anion selective, the influence of potential on Ca\(^{2+}\) influx diminishes, reflecting the decreasing Ca\(^{2+}\) permeability as \( c_{m0} \) increases. The qualitative form of \( \delta \) as a function of \( c_{m0} \) should, therefore, parallel that of the TEC (per unit ionic strength). A comparison of Fig. 8 with Fig. 2B confirms that expectation (see also following text). On that basis, we might expect the form of \( \delta \) to hold also for voltages \( \geq 26 \text{ mV} \) (i.e., for \( v > 1 \)) but with different limits. The average value of \( \delta \) for \( c_{m0} \) between 0 and 0.6 M is 0.15. Accordingly, in fitting the CT responses in Fig. 2A to Eq. 14, \( \delta \) is treated as an adjustable constant, expected to lie between 0.05 and 0.3 and most probably near the average of 0.15.
Fitting the data

To fit the CT response data for CaCl₂, we assume that the response, \( R \), is simply proportional to \( c_i \). Equation 14 can be recast as

\[
c_i = \frac{(3p/\gamma)e^{-2\gamma}}{1 + 2p + \frac{3p}{\gamma}} + \frac{c_m e^{-\gamma}}{k}
\] (15)

The CT response, \( R \), can then be written as

\[
R = \frac{4aD_{Ca}e^{-2\gamma}}{b + \left(\frac{c_m e^{-\gamma}}{k}\right)^n}
\] (16)

where \( a \) is a scaling constant and \( b \) is

\[
b = 1 + 2p + \frac{3p}{\gamma}
\] (17)

It follows that

\[
p = \frac{(b - 1)\gamma}{2\gamma + 3}
\] (18)

indicating that \( b \) must be >1 if negative permeabilities are to be avoided. In fitting the CT responses to Eq. 16, there are as many as five free parameters. The zero current constraint reduces the number to four, still a high degree of freedom. When a four parameter fit was implemented values of 0.37 M and 3.8 were obtained for \( k \) and \( n \), respectively. However, \( b = 0.62 \), i.e., \( <1 \). The value \( n = 3.8 \) agrees well with values obtained by fitting the conductance data in Fig. 2B empirically to a Hill equation. Also in Fig. 2B, the most rapid drop in conductance occurs between 0.1 and 0.3 M, suggesting an estimate of \( k \) of ~0.2 M. If we constrain \( k \) and \( n \) at 0.2 M and 4, respectively, and seek a two-parameter fit, then \( a \) and \( b \) are: 26.8 and 8.64, respectively. This two-parameter fit is displayed in Fig. 2A for the CT response data under zero current clamp and represents the data well. With \( b = 8.64 \), the ratio of the Ca²⁺ permeability coefficient to that of Cl⁻ in the limit of zero CaCl₂, \( p \), is seen to be narrowly constrained. For \( \gamma = 25 \), \( P = 3.6 \), in the limit as \( \gamma \) approaches infinity, \( P = 3.8 \). This is consistent with the value of \( P = 2 \) chosen a priori in plotting the theoretical curves shown in Fig. 8. In fitting the data in Fig. 2A under voltage clamp (±50 mV), we required that the current clamp values of \( a \), \( b \), \( k \), and \( n \) be maintained, and that the fit be achieved by varying the single parameter \( \delta \). The fit shown in Fig. 2A for the clamp voltage, −50 mV, was obtained with \( \delta = 0.12 \), and at +50 mV with \( \delta = 0.11 \), values that are consistent with the range of expected values.

Interpretation of the conductance data

The curve in Fig. 2B of the conductance per unit ionic strength of CaCl₂ was obtained as follows. A perturbation in potential imposed on the open circuit potential (Eq. 3) will produce a small current. Provided the voltage-perturbation is fast compared with diffusional relaxation, the steady state zero-current concentration profiles will prevail. The conductance per unit ionic strength, \( L_u \) (in \( \Omega^{-1} \) mol⁻¹ cm⁻¹), is then

\[
L_u = \frac{2F^2P_2(2P + 1)(x - 1)}{3RT \ln x} + L_n
\] (19)

where \( x = (c_i/c_{in}) \), and \( L_n \) is a nonspecific constant leak conductance per unit ionic strength. The dimensionless conductance (normalized to 0.1 M NaCl), \( L_u \), is

\[
L_u = A \left(\frac{(2P + 1)(x - 1)}{\ln x} + B\right)
\] (20)

where \( A \) and \( B \) may be regarded as scaling constants independent of \( c_m \). However, \( B \) also represents the proportion of the conductance outside the taste-receptive regions. For self-consistency, we require that the conductance data be satisfied by the same parameter values obtained in fitting the CT response curves (Fig. 2A). Accordingly we use Eq. 15 with \( v = 0 \) to calculate \( x \), keeping \( k = 0.2, n = 4 \) and using the least squares fit parameter, \( b = 8.64 \), to calculate, \( p \), the ratio of the Ca²⁺ permeability coefficient to that of Cl⁻ at zero CaCl₂ concentration. Using, as before, \( \gamma = 150 \), constrains, \( p \), to a value of 3.78 (cf. Eq. 18). In Eq. 20, therefore, \( x \) and \( P \) are determined fully and the only parameters remaining are \( A \) and \( B \). Using least-squares criteria on Eq. 20 provides the fit of the conductance-concentration data shown in Fig. 2B with \( A = 0.14 \) and \( B = 0.067 \).

The conductance per unit ionic strength of CaCl₂ is only 30% that of NaCl at maximum (at \( c_m = 0 \)). In this limit, the percent of the total conductance attributable to taste-receptive regions (i.e., the conductance in excess of that of nonspecific leaks) is ~79%. At 0.3 M, it has dropped to ~47%, and by 0.5 M, it is but 27%. As seen in Figs. 1 and 2A, the maximum CT response occurs near 0.3 M. CT responses begin their decline, therefore, when the conductance through the taste-receptive regions falls to <50% of the total conductance. This finding further supports the view that transport of the stimulus across the paracellular regions in the taste buds (as reflected in the conductance) is critical in sustaining the taste response.

Transepithelial potential

The zero current potential, \( \phi_0 \), with \( c_m \) has the expected properties of the system. In Fig. 1, the potential increases in the electronegative direction as the CaCl₂ concentration increases, and this occurs (between 0.1 and 0.3 M) even as the CT response is increasing. The model shows this same characteristic. Normalizing the CaCl₂ potential to that of 0.1 M NaCl gives the dimensionless potential \( \phi_s \) plotted in Fig.

<table>
<thead>
<tr>
<th>( \gamma )</th>
<th>( c_m, \text{mM} )</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
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<tr>
<td>25</td>
<td>7.7</td>
</tr>
<tr>
<td>75</td>
<td>2.6</td>
</tr>
<tr>
<td>150</td>
<td>1.3</td>
</tr>
</tbody>
</table>

The index of diffusion control, \( \gamma (= \alpha VAP) \) is a dimensionless constant characteristic of the system. \( V/A \) is the volume to diffusion surface ratio of the submucosal compartment, \( \alpha \) is the rate constant that determines the clearance of Ca²⁺ from the submucosal compartment, and \( P_2 \) is the chloride permeability coefficient. The larger \( \gamma \), the smaller is the maximum possible value of the CaCl₂ that will occur in the submucosal microenvironment. To place \( c_m \) within reasonable physiological limits, \( \gamma \) must be ≥25 and perhaps as great as 150.
2C as a function log $c_m$. The curve was plotted according to Eq. 3, put in the form

$$\phi_0 = -A_1 \frac{P-1}{2P+1} \ln x + B_1$$  \hspace{1cm} (21)

We have retained all of the parameters used in fitting Figs. 2, A and B, except for the scaling constants $A_1$ and $B_1$, determined as 0.76 and -2.54, respectively. In accord with the data, the changing ionic selectivity of the diffusion barrier therefore is seen in the potential before the submucosal Ca concentration (and therefore the CT response) reaches its maximum. Thus the changes in potential seen with CaCl$_2$ are not, themselves, the cause of the self-inhibition in the CT response induced by CaCl$_2$ but rather the consequence of the cooperative changes in the Ca$^{2+}$ permeability of the paracellular barrier, resulting in increased relative Cl$^{-}$ permeability but overall decreased salt permeability (i.e., conductance, see preceding text).

**DISCUSSION**

**Paracellular Ca$^{2+}$-induced changes in TEP and self-inhibition of CT responses**

The paracellular pathway mediates passive Ca$^{2+}$ transport in various epithelia including intestine (Bronner and Spence 1988), renal (Bronner 1989), and lingual (Sostman and Simon 1991) epithelia. Our data are consistent with that view, i.e., Ca$^{2+}$ permeation of the taste bud epithelium occurs principally through paracellular shunts. The TEP, established across these shunts, is normally moderately cation selective (Berry et al. 1978; DeSimone et al. 1984; Simon et al. 1988; Reuss 1991; Ye et al. 1993), but CaCl$_2$ produces an electronegative change in the TEP (Figs. 1 and 2C). This is consistent with similar observations in small intestine (Smyth and Wright 1966), choroid plexus (Prather and Wright 1969), and gallbladder (Moreno and Diamond 1974).

Ca$^{2+}$ binding to fixed anionic sites will buffer Ca$^{2+}$, initially reducing the effective Ca$^{2+}$ concentration during Ca$^{2+}$ diffusion. However, fixed charge density is limited so buffering will be transient. The major effect of Ca$^{2+}$ binding is reduced Ca$^{2+}$ permeability and consequently reduced CaCl$_2$ conductance (cf. Fig. 2B). The CT response initially increases (e.g., between 0.1 and 0.3 M, cf. Fig. 2A). This suggests that, although the Ca$^{2+}$ permeability is decreasing (cf. Fig. 2B), it is still sufficiently high to cause an increase in the submucosal Ca$^{2+}$ concentration. The model predicts an increase in submucosal Ca$^{2+}$ concentration as stimulus Ca$^{2+}$ concentration increases between 0 and 0.3 M (cf. Fig. 8). However, >0.3 M, the response decreases as the CaCl$_2$ concentration on the mucosal side increases. As seen in Fig. 2B, this coincides with the low CaCl$_2$ conductance regime, where the model indicates (cf. Fig. 8) that increasing submucosal concentrations no longer can be sustained. The CT response (Fig. 2A) and the TEC (Fig. 2B) begin their decline over a narrow range of CaCl$_2$ concentrations, suggesting that both derive from a highly cooperative process. As we have shown, the single assumption of a Ca$^{2+}$ permeability regulated cooperatively by the Ca$^{2+}$ concentration itself is sufficient to account for: the electronegative TEP, the falling TEC, and ultimately the self-inhibiting CT response.

Extracellular Ca$^{2+}$ has been shown to regulate the degree of tightness of the TJs (Contreras et al. 1991), suggesting that the TJs mediate Ca$^{2+}$-induced reduction in CaCl$_2$ permeability. Modulation of TJ properties has been demonstrated in various species including amphibia (Moreno and Diamond 1974), suggesting the nonmonotonic concentration-response relationships for CaCl$_2$, obtained from single-unit recording from amphibian peripheral taste nerves (Kitada 1995), also may arise through paracellular processes.

Although the fibrous material of TJs is not fully characterized (Madara 1991), their ion-exchange (polyelectrolyte) properties are well established. Moreover, polyelectrolyte fibers in model systems, subjected to an ion exchange reaction with Ca$^{2+}$, undergo length changes resembling phase transitions in that the changes occur at critical inducing concentrations as in a highly cooperative process (Katchalsky and Oplatka 1971). In addition, Ca$^{2+}$ and other divalent cations have been shown to induce contraction of the perijunctional actomyosin ring, located just below the TJs (Burgess 1982; Rodewald et al. 1976). If Ca$^{2+}$, through such mechaanochemical transformations, can reduce its own TJ permeability and therefore its own CT response, it is reasonable to expect that it will inhibit the CT responses of other stimuli depending on paracellular transport.

**Ca$^{2+}$-sensitivity of NaCl, KCl, and NH$_4$Cl CT responses**

Part of the NaCl-evoked CT response seems to arise from transduction sites located below the TJs (Bradley 1973; Elliot and Simon 1990; Mierson et al. 1996; Simon et al. 1993; Stewart et al. 1995; Ye et al. 1993). If Ca$^{2+}$ can block access to its own paracellular transduction sites, it should block completely the AIC of the NaCl CT response attributed to transduction sites below the TJs (Elliot and Simon 1990; Mierson et al. 1996; Simon et al. 1993; Stewart et al. 1995; Ye et al. 1993). As seen in Figs. 3 and 4, Ca$^{2+}$ inhibited the CT responses of NaCl. The percentage inhibition was higher at 0.3 M NaCl than at 0.1 M NaCl, corresponding approximately to the percentage of the CT response due to the AICs at these NaCl concentrations (Elliot and Simon 1990; Ye et al. 1993). Ca$^{2+}$ also can inhibit Na$^{+}$ channels, but inhibition constants exceed 0.3 M (Palmer 1986). In mixtures with CaCl$_2$ concentration >0.3 M, some further suppression occurred, probably attributable to some direct inhibition of the apical Na$^{+}$ channels by Ca$^{2+}$. Direct suppression of the Na$^{+}$ channels by Ca$^{2+}$ would not explain the different levels of suppression of 0.1 and 0.3 M NaCl.

Although the magnitude of the NaCl CT response blocked by Ca$^{2+}$ is quantitatively similar to that of the AIC, this is insufficient to prove that they are identical. However, the results of Fig. 5, in which Ca$^{2+}$ block and amiloride block of NaCl CT responses were implemented together, provide strong evidence in favor of this view. They demonstrate that the AIC of NaCl CT responses is entirely Ca$^{2+}$ sensitive. The block occurs rapidly between 0.2 and 0.3 M CaCl$_2$ (cf. Figs. 3 and 4), which coincides with the range over which the conductance declines rapidly as a function of CaCl$_2$ concentration (Fig. 2B), suggesting that Ca$^{2+}$ blocks Na$^{+}$ permeability (and therefore CT response) by the same cooperative process. This is further evidence that taste transduction...
sites for Na⁺ are accessible only by way of a paracellular shunt. Although the AIC comprises <50% of the NaCl-evoked taste intensity in the rat, in human NaCl perception it appears to be more dominant (Ossebaard and Smith 1995). It is unknown, however, if the human AIC is Ca²⁺ sensitive in a manner comparable with rat.

The paracellular pathway also has been implicated in taste transduction for K⁺ salts (Ye et al. 1994). If so, Ca²⁺ should block completely KCl-evoked taste responses. Figure 6 demonstrates that 0.3 M KCl CT responses, which occurred in mixtures with CaCl₂ ≥0.3 M, were eliminated completely. These results support the view that KCl transduction is mediated largely through a paracellular pathway susceptible to block by Ca²⁺.

Earlier work shows that <0.3 M, NH₄Cl mainly uses a transcellular taste transduction pathway, whereas, ≥0.3 M, a paracellular pathway is favored (Kloub et al. 1997). Therefore if the Ca²⁺ effects observed here are exerted mainly on the ion permeability of the TJs, Ca²⁺ block of the NH₄Cl CT response should be most effective at higher NH₄Cl concentrations. This is demonstrated in Fig. 7. The differential effect of CaCl₂ on the two concentration domains of the NH₄Cl CT response reinforces the earlier conclusions regarding dual transduction mechanisms for NH₄Cl, with the one predominating at higher NH₄⁺ concentrations being principally shunt-mediated.

Concentration modulator properties of the paracellular diffusion barrier

The concentration range of salt taste sensitivity is typically from millimolar to hundreds of millimolar. Changes of this order in the intercellular milieu almost certainly would be injurious to the sensory cells. In the case of CaCl₂, exceeding the intracellular buffering capacity for Ca²⁺ would lead to cell death (McConkey and Orrenius 1996). Ca²⁺-induced inhibition of its own CT response and that of other salts is reversible and without obvious cytotoxicity, suggesting that Ca²⁺ concentrations at the effector sites are far below those in the applied stimulus solution. We conclude that CaCl₂ concentration attenuation is achieved by placing the common transduction pathway for Ca²⁺, K⁺, Na⁺, and NH₄⁺ salts in series with a significant diffusion barrier in a manner demonstrated in the analysis. Ca²⁺, of course, is also an important intracellular regulatory agent. Modulation of TJ permeability in various epithelial and endothelial tissues may be subject therefore to physiological regulation by Ca²⁺ supplied to the submucosal microenvironment from internal stores.

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Kloub, G. L., Heck, G. L., and DeSimone, J. A. A comparative study of amiloride inhibition of chorda tympani taste responses under lingual voltage clamp: implications for NH₄ salt taste concentration attenuation is achieved by placing the common transduction pathway for Ca²⁺, K⁺, Na⁺, and NH₄⁺ salts in series with a significant diffusion barrier in a manner demonstrated in the analysis. Ca²⁺, of course, is also an important intracellular regulatory agent. Modulation of TJ permeability in various epithelial and endothelial tissues may be subject therefore to physiological regulation by Ca²⁺ supplied to the submucosal microenvironment from internal stores.

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