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Hypoosmotic Stimuli Activate a Chloride Conductance in Rat Taste Cells

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Abstract

The oral cavity is subjected to a wide range of osmotic conditions, yet little is known about how solution osmolarity affects performance of the gustatory system. In order to elucidate the mechanism by which hypoosmotic stimuli affect the peripheral taste system, I have attempted to characterize the effects of hypoosmotic stimuli on individual rat taste receptor cells (TRCs) using whole-cell patch clamp recording. Currents elicited in response to voltage ramps (–90 to +60 mV) were recorded in control saline and in solutions varying only in osmolarity (–30, –60 and –90 mOsm). In roughly two-thirds of cells, hypoosmotic solutions (230 mOsm) caused a 15% increase in cell capacitance and activated a reversible conductance that exhibited marked adaptation in the continued presence of the stimulus. Similar responses could be elicited in taste cells from taste buds in the foliate and vallate papillae, the soft palate, the nasopharynx and the epiglottis. Ion substitution experiments were consistent with the interpretation that the predominant ion carried through these apparent volume- or stretch-activated channels was Cl^- under normal conditions. Reversal potentials for the hypoosmotic-induced current closely matched those predicted by the Goldman–Hodgkin–Katz constant field equation for a Cl^- conductance. The relative permeability sequence of the hypoosmotic-activated current in TRCs was thiocyanate$^-$ ≥ I^- ≥ Br^- > Cl^- ≥ F^- ≥ isethionate$^-$ > gluconate$^-$. Pharmacological experiments revealed that this Cl^- conductance was inhibited by 4,4′-diisothiocyanatostilbene-2, 2′-disulfonic acid and 5-nitro-3-(3-phenylpropylamino)benzoic acid (EC_{50} = 1.3 and 4.6 μM, respectively), but not by $CdCl_2$ (300 μM) nor $GdCl_3$ (200 μM). I hypothesize that this hypoosmotic-activated Cl^- conductance, which is similar to the well-characterized swelling-activated Cl^- current, may contribute to volume regulation and could represent the transduction mechanism by which the presence of hypoosmotic stimuli, including water, may be signaled in taste receptor cells.

Introduction

Taste receptor cells (TRCs) respond to a variety of sapid molecules corresponding to the basic human perceptual correlates of salty, sour, sweet, bitter and umami. The molecules that comprise tastants are varied and encompass such chemicals as small ions up to complex organic molecules that are common to many bitter and sweet taste stimuli (Gilbertson and Kinnamon, 1996). Accordingly, the mechanisms that have been elucidated for the transduction of taste stimuli are varied and include direct interaction with ion channels and activation of ionotropic and metabotropic receptors [for reviews see (Lindemann, 1996; Gilbertson *et al*., 2000)]. TRCs also respond to a variety of compounds outside the realm of the basic tastes that serve to modulate the response of the receptor cell to other tastants. A classic example of this phenomenon is the enhancement of TRC responses to monosodium glutamate by 5′-ribonucleotides (Ninomiya *et al*., 1992). Additional factors that have been reported to modify gustatory responses at the level of the receptor cell include astringents (Schiffmann *et al*., 1992), divalent cations (Myers *et al*., 1993), anions (Gilbertson *et al*., 1997a), polyunsaturated fatty acids (Gilbertson *et al*., 1997b; Gilbertson, 1998), other lipids (Schiffman *et al*., 1995) and O_2 availability (Singh *et al*., 1997).

An additional factor that has received comparatively little attention is the osmotic status of the taste stimulus. Taste stimuli range from the very hypoosmotic to those an order of magnitude greater than the osmolarity of the taste cell interior milieu (Feldman and Barnett, 1995). Much of our current understanding about the transduction mechanisms in mammalian TRCs has come from experiments in which isolated taste buds are recorded from using electrophysiological or calcium imaging techniques. In these experiments, taste stimuli are typically presented in an isosmotic solution, a condition not always the case *in vivo*. Recently, Lyall and colleagues (Lyall *et al.*, 1999) demonstrated that hyperosmotic stimuli enhance rat chorda tympani responses to NaCl, thereby demonstrating that changes in TRC volume can affect tastant-induced activity in the peripheral gustatory system.

Higher vertebrates require that intracellular and extracellular osmolarity be well controlled, and differences between the intracellular and extracellular osmolarities of >1% can often lead to the activation of compensatory

mechanisms (Wenning, 1999). Most cells when exposed to hypoosmotic stimuli have mechanisms to compensate for the osmotic difference and increase their cell volumes in response. Typically, these cells allow the influx of water through aquaporins (AQPs) (Echevarría and Ilundáin, 1998; Heymann and Engel, 1999) followed by a transient or sustained increase in cell volume. For cells like aortic baroreceptors (Cunningham *et al*., 1995), cardiac myocytes (Hall *et al*., 1997; Köhler *et al*., 1998) and central nervous system neurons (Law, 1996) that often are exposed to changes in extracellular osmolarity, this increase in cell volume is sensed by corresponding changes in the activity of mechanosensitive ion channels. These mechanosensitive channels include those that have been characterized as stretch-activated, stretch-inactivated and pressure-sensitive (Morris, 1990), and have ionic permeabilities that include Cl^-, K^+ or Ca^{2+}.

Little is known about how taste cells respond to hypoosmotic changes on either the apical or basolateral membranes and how this might affect their ability to respond to taste stimuli. Recently, my laboratory demonstrated the presence of three varieties of AQPs in taste cells using immunocytochemistry and reverse transcriptase–polymerase chain reaction (Kim *et al*., 1999). Interestingly, aquaporin-5 (AQP-5) was apparently restricted to the apical membranes, while two other AQPs, AQP-1 and AQP-2, were present predominantly on the basolateral membrane. Thus, there are the molecular components present in TRCs to allow rapid water entry and to compensate for changes in osmolarity. In the present study, I have investigated the effects of hypoosmotic stimuli on isolated rat taste buds using whole-cell patch clamp recording. Consistent with other osmotic-sensitive cells, TRCs responded to hypoosmotic stimuli by increases in cell volume, as measured by changes in cell capacitance, and the subsequent activation of stretch-sensitive Cl^- channels. This mechanism may have implications both for the response to hypoosmotic taste stimuli and in the gustatory response to water (Cohen *et al*., 1955; Ishiko and Sato, 1973; Sato *et al*., 1995; Lindemann, 1996).

Part of these results has appeared in abstract form (Gilbertson *et al*., 2000).

Materials and methods

Taste bud isolation and patch clamp recording

Individual fungiform taste buds were isolated from the tongues of 2- to 5-month-old male Sprague–Dawley rats using techniques described previously (Béhé *et al*., 1990; Doolin and Gilbertson, 1996). Briefly, tongues were isolated and injected between the muscle layer and the lingual epithelium with ~1.0 ml of physiological saline (Tyrode) containing a mixture of collagenase I (0.5 mg/ml; Boeringer Mannheim, Indianapolis, IN), dispase (5 mg/ml; Boeringer Mannheim) and trypsin inhibitor (1 mg/ml; type I-S; Sigma Chemical Corp., St Louis, MO). The injected tongue was incubated in a Ca^{2+}–Mg^{2+}-free Tyrode containing 2 mM glycine, *N*, *N*′-(1,2-ethanediylbis(oxy-2, 1-phenylene))bis(*N*-(carboxymethyl))-tetrapotassium salt (BAPTA, Molecular Probes Inc., Eugene, OR) and bubbled with O_2 for 25 min at room temperature. Following incubation, the tongue was washed with saline, and the epithelium was removed from the underlying muscle layer with forceps and pinned out in a Sylgard™-lined Petri dish. Individual taste buds were removed from the epithelium under low magnification (×50) with a suction pipette (~200 μm pore) and plated out into the recording chamber. In some experiments, taste buds were isolated from the foliate and vallate papillae (Doolin and Gilbertson, 1996), soft palate, nasopharynx and epiglottis using methods similar to those used for other non-lingual taste buds (Gilbertson and Fontenot, 1998). The recording chamber consisted of a Cell-Tak Tissue Adhesive (Boehringer Mannheim) coated microscope slide fitted with an O-ring. Though all taste bud types responded to decreases in osmolarity of extracellular solutions in a qualitatively similar fashion (cf. Figures 1 and 2), the majority of the present work focused on the response in taste cells from the fungiform papillae. The criteria used to establish a cell as a taste receptor cell and not an epithelial cell was the same as used in previous work (Gilbertson *et al*. 1993; Gilbertson and Fontenot, 1998). Once in the recording chamber, cells were perfused with extracellular solution (Tyrode) containing (in mM): 140 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid (HEPES), 10 glucose and 10 Na^+ pyruvate (~310 mOsm). The pH was adjusted to 7.4 with NaOH. In the initial experiments, a standard intracellular (pipette) solution was used that contained (in mM): 140 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, 11 ethylene glycol-bis(-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) and 3 ATP. The pH was adjusted to 7.2 with KOH (~310 mOsm). In most experiments, however, a low K^+ intracellular solution was used, in which 90 mM CsCl and 100 mM mannitol were substituted for the KCl and the pH was adjusted to 7.2 with CsOH (~310 mOsm). This CsCl intracellular solution helped to eliminate most of the voltage-activated outward K^+ current, which facilitated the analysis of the hypoosmotic-induced current and set the Cl^- equilibrium potential (E_{Cl}) to near zero. Nonetheless, the results obtained were equivalent regardless of the intracellular solution used.

Voltage-activated currents were recorded from individual taste receptor cells (TRCs) maintained in the taste bud by using the whole-cell patch clamp configuration. Patch pipettes were pulled to a resistance of 5–10 MΩ when filled with intracellular solution. Series resistance and cell capacitance were compensated optimally before the recording. The holding potential in all experiments was –80 mV. In most cases, ramp protocols from –90 mV to +60 mV (480 ms duration, 0.31 V/s) were used to generate instantaneous current–voltage (*I–V*) relationships in the various solutions.

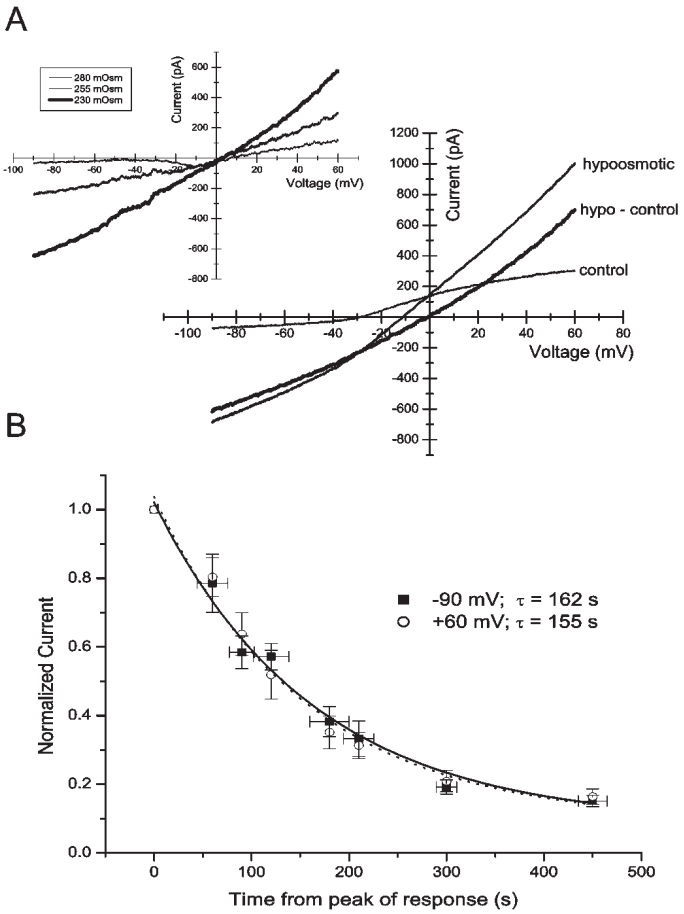

Figure 1 Hypoosmotic stimuli activate an outwardly rectifying conductance in taste cells. **(A)** Currents elicited in response to voltage ramps from –90 to +60 mV in control (standard, Table 1) and hypoosmotic solutions in a rat taste cell from the fungiform papillae. The hypoosmotic minus control (hypo – control) trace reflects the net current elicited by the hypoosmotic stimuli (I_{HYPO-T}). Inset, magnitude of hypoosmotic-induced currents is inversely correlated with osmolarity. Net hypoosmotic-induced currents were elicited in three solutions varying in osmolarity, including 280 mOsm (thin line), 255 mOsm (medium line) and 230 mOsm (thick line), with an intracellular solution of 310 mOsm. All current traces shown reflect the peak of the response to the hypoosmotic stimuli. **(B)** Hypoosmotic-induced currents adapt in the continued presence of hypoosmotic stimuli. Plot shows the decay of the mean currents at +60 mV (■) and at –90 mV (○) versus time from the peak of the response at time 0. The nearly superimposed solid and dotted lines are the best fits with single exponential functions to the –90 mV data and the +60 mV datasets, respectively. The time constants for the adaptation (τ) were 162 s at –90 mV and 155 s at +60 mV.

Command potentials were delivered and current data were recorded with pCLAMP software (versions 7/8) interfaced to an AxoPatch 200A or 200B amplifier with a Digidata 1200A A/D board (Axon Instruments, Foster City, CA). Data were collected at 10 kHz and filtered online at 2 kHz. No records were leak subtracted in the present study.

Solutions

To elicit hypoosmotic responses in TRCs, solutions were prepared that varied only in osmolarity from the control solution. A control solution was prepared that contained 100 mM mannitol, and hypoosmotic solutions were made by decreasing the total mannitol concentration (to 10, 40 or 70 mM; Table 1). Thus, the ionic components of all solutions were kept constant. The control solution contained 90 mM NaCl and substitution for the Cl^- with Na salts containing other anions (Table 1) was used in experiments designed to determine the relative permeability of the hypoosmotic-induced current. Junction potentials were determined empirically for all solutions and reversal potentials were corrected for junction potentials prior to analysis. The osmolarity of all solutions used in the present study was measured with a vapor pressure osmometer (Model 5500, Wescor, Logan, UT) and adjusted, if necessary, with water or mannitol.

The pharmacology of the hypoosmotic-induced current was examined using a number of Cl^- channel blockers known to target volume- or stretch-activated conductances (Quasthoff, 1994; Xu *et al.*, 1997; Hume *et al.*, 2000; Pérez-Samartín *et al.*, 2000), including DIDS (4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid), $CdCl_2$, $GdCl_3$ and NPPB [5-nitro-3-(3-phenylpropylamino)benzoic acid]. These inhibitors were added to both the control (310 mOsm) and hypoosmotic (230 mOsm) solutions, and applied by bath perfusion. The effectiveness of these inhibitors was determined by measuring the net hypoosmotic-induced current in the absence and presence of the inhibitors at the peak of the response at +60 mV. This potential was chosen to eliminate the complication of the strong voltage-dependence of Cl^- channel inhibition by DIDS (Xu *et al.*, 1997) (Figure 5A). Relative inhibition was calculated as the ratio of the peak current in the presence of the inhibitor over the current in the absence of the inhibitor.

Data analysis

To isolate the hypoosmotic-induced current, the control traces (e.g. *I–V* curves) that immediately preceded the experimental condition were subtracted from those in the presence of hypoosmotic stimuli. The traces selected to reflect the experimental condition were those that were collected during the peak of the response to the hypoosmotic stimuli. Data were collected every 30–60 s during the control, experimental and recovery conditions. In general, the peak of the response to hypoosmotic stimulation occurred ~1–3 min after solution change. This peak value was determined empirically for each cell used for the analysis. Because the responses to hypoosmotic stimuli adapted significantly (see Figure 1B) and I could not be sure the properties of the adapted response were equivalent to those prior to adaptation, I was careful to choose responses that represented the peak of the current response for subsequent analysis.

Statistical analysis was performed using SPSS software (v. 7.5, SPSS Inc., Chicago, IL) with the level of significance

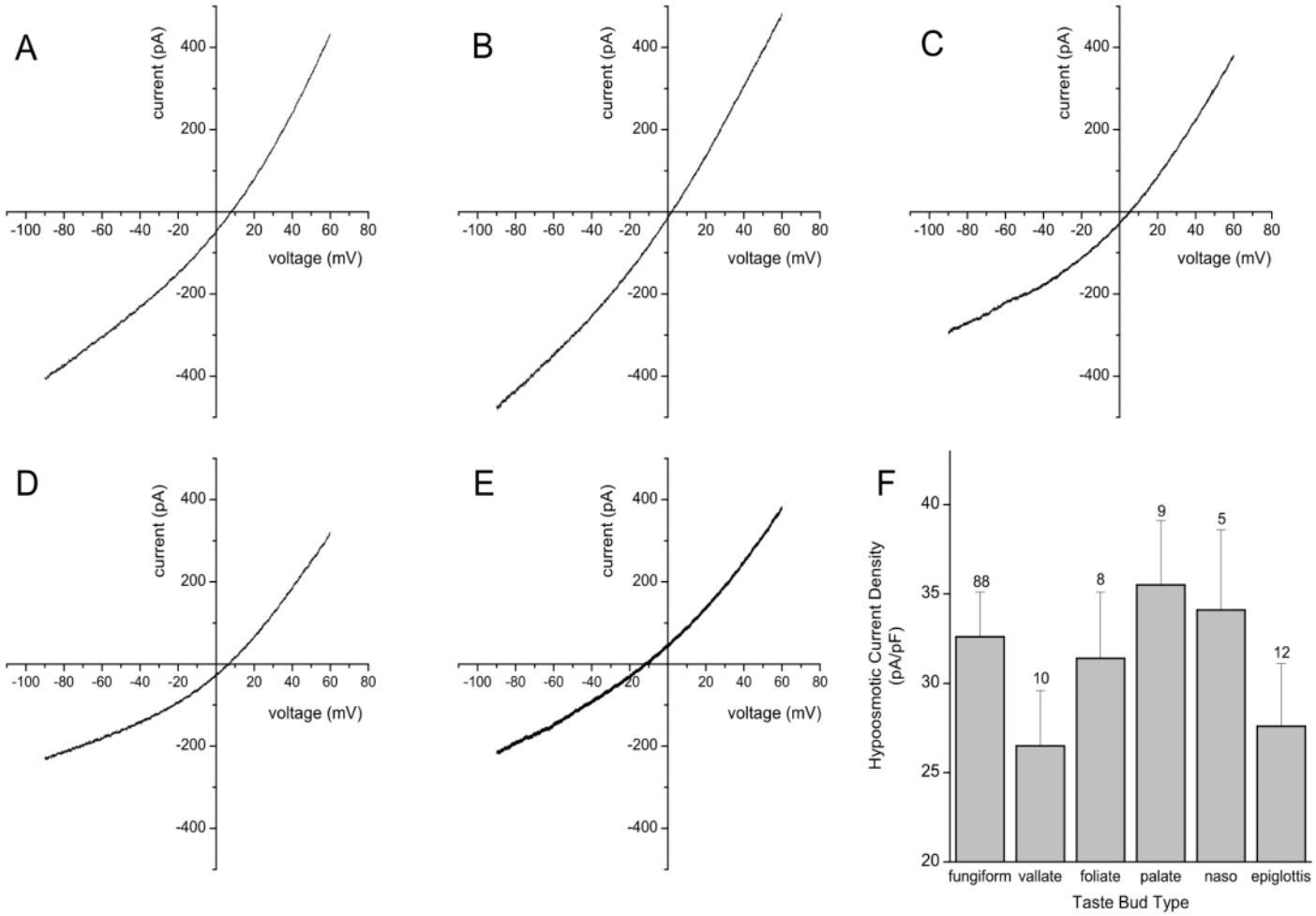

Figure 2 Hypoosmotic-induced currents can be elicited from a variety of taste bud types. Net hypoosmotic currents are shown in taste buds from the vallate **(A)** and foliate **(B)** papillae of the tongue, the soft palate **(C)**, the nasopharynx **(D)** and the epiglottis **(E)**. These responses appeared qualitatively similar to those seen on the fungiform taste buds (Figure 1) in terms of both current magnitude and permeability properties (zero current potential in standard solutions). **(F)** Current density of the hypoosmotic-induced responses in the various types of taste cells. Values shown are means ± SD, and the numbers above the bars are the number of cells included in the analysis. One-way ANOVA revealed no significant differences were present among the various taste bud classes ($F = 0.864$, df = 5, $P = 0.51$).

(α) set at 0.05 in all cases. One-way analyses of variance (ANOVAs) were conducted to determine the differences among the magnitude of current densities induced by hypoosmotic stimuli in the various taste bud types and to determine the significant differences in the relative permeabilities for the anions carrying the hypoosmotic-induced current. Specific details are given in the text.

The Institutional Animal Care and Use Committees of Pennington Biomedical Research Center and Utah State University approved all procedures involving animals.

Results

Effects of hypoosmotic stimuli on taste receptor cells

To examine the effects of hypoosmotic stimuli on taste receptor cells, I used whole-cell patch clamping on taste buds isolated from the tongue and oral cavity in 2- to 5-month-old male Sprague–Dawley rats. Control currents were elicited in an isotonic saline (310 mOsm) in response to voltage ramps from –90 to +60 mV (0.31 V/s) and displayed a typically outwardly rectifying waveform when a high K^+ intracellular solution was used (Figure 1A). Changing to a hypoosmotic (230 mOsm) extracellular solution (Table 1) led to a pronounced increase in conductance in response to the voltage ramp in roughly two-thirds of fungiform taste cells (61.1%, $n = 144$ cells). This change was evident within a few seconds following solution change and continued to increase in magnitude for 1 to several min (see below). The remaining cells showed little or no response to the hypoosmotic stimulus. To isolate the net hypoosmotic-induced current in taste cells ($I_{HYPO\text{-}T}$) the control I–V curve was subtracted from that generated in the presence of hypoosmotic stimuli (Figure 1A). Examination of $I_{HYPO\text{-}T}$ showed that it had a weakly outwardly rectifying waveform

Table 1 Composition of standard and hypoosmotic solutions

Component	Standard	Hypo-osmotic	Low Cl^- standard	Hypo-osmotic low Cl^-
NaCl	90	90	0	0
Na-anion[a]	0	0	90	90
Mannitol	100	10[b]	100	10
KCl	5	5	5	5
$CaCl_2$	1	1	1	1
$MgCl_2$	1	1	1	1
HEPES	10	10	10	10
Glucose	10	10	10	10
Na-pyruvate	10	10	10	10
Osmolarity (mOsm)	310 ± 3	230 ± 3	310 ± 3	230 ± 3

All concentrations are given in mM. Osmolarity was measured with a vapor pressure osmometer and adjusted, if necessary, with mannitol or water.

[a]Sodium salts of the following anions were used in the studies designed to determine the anionic permeability of the hypoosmotic-activated conductance: bromide, iodide, thiocyanate, fluoride, isethionate, gluconate. Each of these salts was substituted equimolar for NaCl.

[b]In some experiments (cf. Figure 1), 40 or 70 mM mannitol was used to create hypoosmotic solutions with osmolarities of 255 and 280 mOsm, respectively.

with a reversal potential near 0 mV with equimolar Cl^- on the two sides of the membrane.

As shown in Figure 1A, the magnitude of $I_{HYPO\text{-}T}$ increased with decreasing extracellular osmolarity. Concomitant with the increase in whole-cell conductance, hypoosmotic stimuli also lead to an increase in whole-cell membrane capacitance, which reflects a change in membrane surface area (Thiele *et al.*, 1998). In normal saline the mean capacitance of fungiform taste receptor cells in the present study was 9.7 ± 0.5 pF (n = 42 cells); when placed into hypoosmotic saline the capacitance increased significantly to 11.1 ± 0.6 pF (n = 42 cells, $P < 0.0001$, Student's t-test), an increase of 14.9%. The change in membrane capacitance was correlated with the presence of the hypoosmotic-induced current. Cells that showed no significant $I_{HYPO\text{-}T}$ upon stimulation with hypoosmotic stimuli also did not have a significant change in their membrane capacitance (data not shown).

Though the initial response to the hypoosmotic stimulus was rapid (within a few seconds after complete solution change), the magnitude of $I_{HYPO\text{-}T}$ did not reach a maximum until ~1–3 min after changing solutions from the control to hypoosmotic solutions (the time for complete solution change in the chamber used was <5 s). Following its maximal activation, $I_{HYPO\text{-}T}$ began to decay despite the continued presence of the hypoosmotic stimulus. Figure 1B shows the decay of $I_{HYPO\text{-}T}$ as a function of time following the peak of the response. Within ~5 min , $I_{HYPO\text{-}T}$ decayed to ~20% of its maximum value. There was little or no voltage dependence to this decay since time constants (τ) measured at +60 (τ_{+60} = 162 s) were not significantly different from those measured at –90 mV (τ_{-90} = 155 s). Adaptation was also seen in the measurements of membrane capacitance. As $I_{HYPO\text{-}T}$ adapted to near prestimulus levels, the change in capacitance associated with the hypoosmotic stimulus also returned close to control levels in most cells (data not shown).

Hypoosmotic stimuli affect many types of taste buds in the oral cavity

In addition to investigating hypoosmotic responses of fungiform taste receptor cells, I also examined the effects of hypoosmotic stimuli on taste cells maintained in isolated taste buds from the foliate and vallate papillae of the tongue and from the soft palate, nasopharynx and epiglottis. In all taste bud types, hypoosmotic stimuli activated a conductance in a subset of cells that looked qualitatively similar to that seen in the fungiform taste receptor cells (Figure 2A–E). Recordings were taken from too few of the other classes of taste buds in the present study to make a detailed analysis meaningful. Nonetheless, there was a limited degree of variability among the various taste bud types in terms of percent responsive cells (fungiform: 61.1%; vallate: 62.5%; foliate: 72.7%; palate: 56.3%; nasopharynx: 41.7%; epiglottis: 85.7%). The current density (pA/pF) of $I_{HYPO\text{-}T}$ showed no significant differences across taste bud types (Figure 2F; P = 0.51, F = 0.865, df = 5).

$I_{HYPO\text{-}T}$ is a Cl^- current

As shown in Figures 1 and 2, the reversal potential for $I_{HYPO\text{-}T}$ was close to zero with equimolar Cl^- on both sides of the membrane in all cell types examined. Because of this and because complete replacement of extracellular NaCl with *N*-methyl-D-glucamine Cl did not appreciably alter the response to hypoosmotic stimuli (data not shown), it was hypothesized that $I_{HYPO\text{-}T}$ might be carried largely by Cl^- ions. To test the involvement of Cl^- in the hypoosmotic-induced response, Na gluconate was substituted for NaCl either partially (60 mM Na gluconate; 30 mM NaCl) or completely (90 mM Na gluconate; 0 mM NaCl) in the hypoosmotic solution listed in Table 1. Consistent with Cl^- contributing to $I_{HYPO\text{-}T}$, changes in extracellular Cl^- activity (concentration × activity coefficient) led to changes in the reversal potential of $I_{HYPO\text{-}T}$ that were closely predicted by the Nernst equation for a pure Cl- conductance ($E_{Cl} = RT/F \ln[Cl]_i/[Cl]_o$; Figure 3). Taking into account the contribution of the relative permeability for gluconate determined empirically (see below and Table 2) I applied the Goldman–Hodgkin–Katz (GHK) equation in the following form:

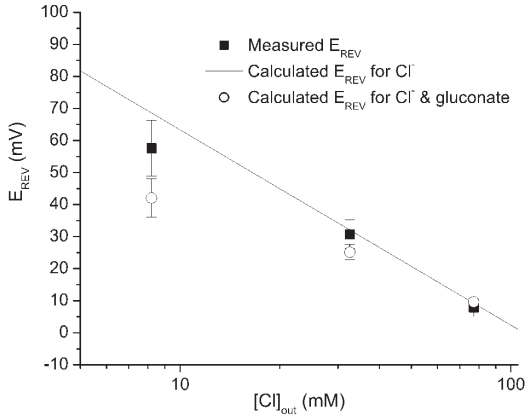

Figure 3 Relationship between reversal potential for the hypoosmotic-induced current and Cl^- concentration is closely predicted by the Nernst equation for a Cl^- conductance. Values for the measured E_{REV} (■) are means ± SD, and represent 12–41 cells per point. The solid line is the relationship predicted by the Nernst equation for a pure Cl^- conductance with a slope of –61 mV/decade. Open circles represent the calculated E_{REV} for both a Cl^- and gluconate$^-$ permeable conductance (P_{gluc}/P_{Cl} = 0.2 ± 0.08; Table 2) using the GHK equation (equation 1). Values for the concentrations of Cl^- have been adjusted to take into account the activity coefficient (λ) of NaCl (99 mM: λ = 0.78; 39 mM: λ = 0.835; 9 mM: λ = 0.91 at 25C) (Lyubartsev and Laaksonen, 1997). Similar values were used for the activity coefficient of Na gluconate (Robinson and Stokes, 1955). Activity coefficients used in the calculation of Cl^- activities in the intracellular solution were as follows: KCl, λ = 0.75; $CaCl_2$, λ = 0.888; $MgCl_2$, λ = 0.852. The reversal potentials have been corrected for errors due to junction potentials in each of the three solutions shown.

$$E_{rev} = \frac{RT}{F} \ln \frac{\left(P_{Cl}[Cl]_i + P_{anion}[anion]_i\right)}{\left(P_{Cl}[Cl]_o + P_{anion}[anion]_o\right)} \quad (1)$$

where R, T and F have their usual thermodynamic meanings. P_{Cl}, P_{anion} refer to the permeability of Cl^- and the anion gluconate$^-$, respectively. [Cl] and [anion] represent the activities of Cl^- and the anion (gluconate$^-$ in this case) in the intracellular (i) and extracellular (o) solutions. The open circles in Figure 3 show the result of applying this form of the GHK equation using a relative permeability of gluconate ($P_{gluconate}/P_{Cl}$) of 0.2 ± 0.08 (Table 2) to the measured reversal potentials for $I_{HYPO\text{-}T}$ in the various solutions.

Anion permeability of the hypoosmotic-induced conductance

To examine the anion permeability of $I_{HYPO\text{-}T}$, extracellular NaCl was replaced with sodium salts containing various anions in both the control and hypoosmotic (230 mOsm) solutions (Table 1). Reversal potentials of $I_{HYPO\text{-}T}$ were measured at the peak of the response (i.e. before the onset of adaptation) to the hypoosmotic stimulus during voltage ramps from –90 to +60 mV. For each cell included in this analysis, reversal potentials were measured in Cl^--containing extracellular solution and from 2–3 other solutions that had equimolar replacement of Cl^- with one of the following anions: thiocyanate (SCN^-), I^-, Br^-, F^-, isethionate$^-$ or gluconate$^-$. Figure 4 shows typical *I–V* curves generated in the presence of Cl^- and four other solutions in which the Cl^- was replaced with SCN^-, I^-, Br^- and isethionate$^-$. All reversal potentials were corrected for errors due to pipette junction potentials. Mean reversal potentials obtained in the various anionic solutions are listed in Table 2. Assuming that $I_{HYPO\text{-}T}$ was carried solely by anions and that the intracellular Cl^- concentration did not change significantly during the hypoosmotic response, the relative permeabilities for the replacement anions (P_x/P_{Cl}) were calculated from the averaged reversal potentials (E_{rev}) by the GHK equation (equation 1). The relative permeabilities for the various anions through the hypoosmotic-activated channel are listed in Table 2. The statistical comparison among the relative permeabilities for the various anionic solutions was per-

Table 2 Effects of replacing Cl^- with other anions on the reversal potential of the hypoosmotic-induced current

Anion	E_{rev} (mV)	P_x/P_{Cl}	*n*
SCN^-	–16.44 ± 1.90	1.92 ± 0.14	10
I^-	–13.40 ± 1.54	1.72 ± 0.10	10
Br^-	–9.65 ± 1.62	1.62 ± 0.09	8
Cl^-	0.90 ± 0.76	1	22
F^-	6.13 ± 1.17	0.79 ± 0.04	8
Isethionate$^-$	12.39 ± 2.36	0.64 ± 0.07	9
Gluconate$^-$	42.69 ± 3.10	0.20 ± 0.08	8

Reversal potentials (E_{rev}) were determined from *I–V* curves generated during the maximum current obtained during hypoosmotic stimulation. Values shown are means ± SEM. P_x/P_{Cl} was calculated from the measured E_{rev} for each anion using the GHK equation (equation 1). P_{Cl} was assumed to be 1.

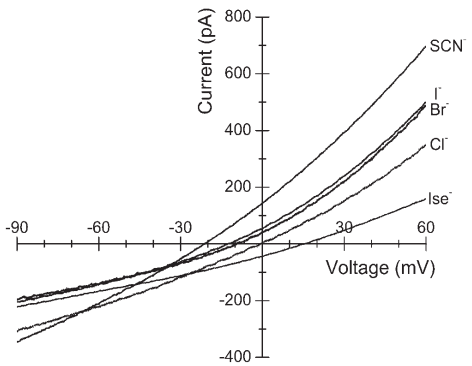

Figure 4 Effects of anion substitution on *I–V* relations of hypoosmotic-induced currents. Peak current responses are shown in response to voltage ramps from –90 to +60 mV (0.31 V/s) applied to fungiform taste receptor cells. Cells were perfused with control and hypoosmotic solutions in which the NaCl was replaced with the Na salts containing the anions shown on an equimolar basis (Table 1). Curves of this type were used to measure the reversal potential of the hypoosmotic-induced current in each solution. The SCN^-, I^- and Br^- traces were from one cell; the Cl^- and isethionate (Ise^-) traces shown were from a different cell.

formed using Tukey's HSD post-hoc test following a one-way ANOVA. Table 3 lists the levels of significance obtained in this analysis (SPSS 7.5, SPSS Inc., Chicago, IL) and reveals a permeability sequence for I_{HYPO-T} as follows:

$$SCN^- \geq I^- \geq Br^- > Cl^- \geq F^- \geq \text{isethionate}^- > \text{gluconate}^-$$

Effects of Cl- channel inhibitors on hypoosmotic-induced currents

The effects of several Cl^- channel inhibitors and blockers of stretch-activated Cl^- channels on I_{HYPO-T} were examined. Both $GdCl_3$ (200 μM) and $CdCl_2$ (300 μM), two known inhibitors of stretch-activated Cl^- channels (Quasthoff, 1994; Pérez-Samartín *et al*., 2000), had no significant effect on hypoosmotic-induced currents in fungiform TRCs (Figure 5A,B). Two other Cl^- channel inhibitors significantly blocked currents though the hypoosmotic-activated channels. DIDS (200 μM) and NPPB (100 μM) inhibited >90% of I_{HYPO-T} measured at +60 mV (Figure 5B). Consistent with other reports (Xu *et al*., 1997), the inhibition by DIDS was significantly voltage dependent. While 200 μM DIDS inhibited 96 ± 2.1% of I_{HYPO-T} at +60 mV, it only inhibited 28 ± 11% of the same current measured at –90 mV (n = 18). NPPB showed no similar voltage dependence, inhibiting roughly equal proportions of I_{HYPO-T} at all potentials. Concentration–response functions were generated for DIDS (0.02–200 μM) and NPPB (0.01–100 μM) for their ability to inhibit I_{HYPO-T} measured at its maximal activation at +60 mV. These inhibitors blocked I_{HYPO-T} in a concentration-dependent manner, and best fits to the data with a logistic function (Origin 6.1, OriginLab Corp., Northampton, MA) showed that the EC_{50} for DIDS was 1.3 μM and for NPPB, 4.6 μM (Figure 5C).

Discussion

During feeding, the peripheral gustatory response reflects not only the response to the individual tastants (i.e. salty,

Table 3 Statistical comparison of the relative permeabilities of I_{HYPO-T} for the various anions

	SCN	I	Br	Cl	F	Ise	Gluc
SCN	–						
I	0.424	–					
Br	*0.002*	0.302	–				
Cl	*<0.001*	*<0.001*	*<0.001*	–			
F	*<0.001*	*<0.001*	*<0.001*	0.501	–		
Ise	*<0.001*	*<0.001*	*<0.001*	*0.012*	0.848	–	
Gluc	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.004*	–

Values represent the level of significance determined by Tukey's HSD post-hoc test following a one-way ANOVA. Values listed in italics denote significant differences at an α level of 0.05. Statistical analysis reveals a permeability sequence for the hypoosmotic-induced current of $SCN^- \geq I^- \geq Br^- > Cl^- \geq F^- \geq Ise^- > Gluc^-$. Ise, isethionate; Gluc, gluconate.

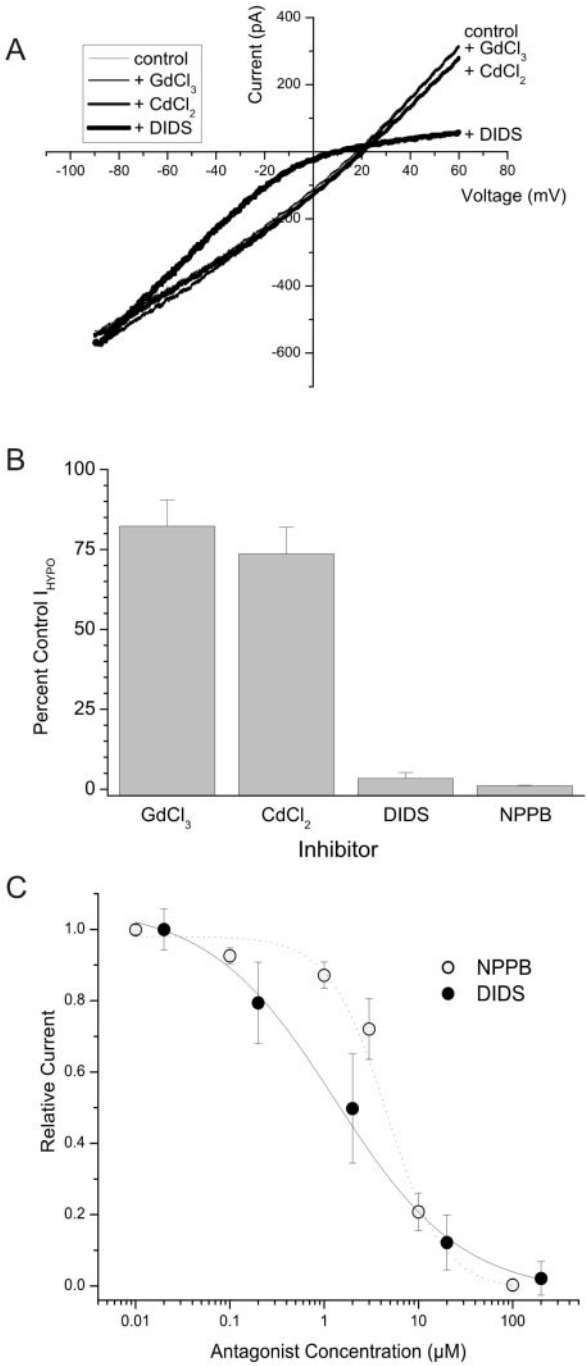

Figure 5 Pharmacology of the hypoosmotic-induced current (I_{HYPO-T}) in taste receptor cells. **(A)** Net hypoosmotic-induced *I–V* curves elicited by 230 mOsm solution under control conditions and in the presence of $GdCl_3$ (200 μM), $CdCl_2$ (300 μM) and DIDS (20 μM) in a single taste receptor cell. Note the voltage dependence of inhibition by DIDS. **(B)** Relative inhibition of hypoosmotic-induced current by several Cl^- channel antagonists. The hypoosmotic-induced currents were measured at their peak at +60 mV and values shown are means ± SEM. Antagonist concentrations used were $GdCl_3$, 200 μM; $CdCl_2$, 300 μM; DIDS, 200 μM; NPPB, 100 μM. **(C)** Concentration–response functions for the relative inhibition of hypoosmotic-induced currents by NPPB and DIDS. Values shown are means ± SD, and represent 8–21 cells per point. Dotted and solid lines are the best fits to the NPPB and DIDS data, respectively, using a logistic function. The EC_{50} value for NPPB was 4.6 μM and for DIDS, 1.3 μM.

sour, bitter, sweet, umami) that may be contained in food, but also the context in which it is presented. Such factors as ionic composition of the saliva, the presence of molecules that may act as taste modulators or those with additional sensory cues (e.g. texture, temperature) may all affect the response generated in the TRCs and carried forward to the gustatory afferents. In the present study, I have investigated the effects of hypoosmotic stimuli on the activity of taste receptor cells using whole-cell patch clamp recording. By altering only the osmotic composition of the stimuli through changes in mannitol concentration and keeping all ionic constituents constant, I have attempted to isolate the response attributable to decreasing osmolarity of the extracellular solution. In roughly two-thirds of cells examined, hypoosmotic stimuli (230–280 mOsm) caused an increase in the membrane capacitance of the TRC and an increase in whole-cell conductance that showed marked adaptation over several minutes of exposure. The increase in conductance was attributable to the activation of a DIDS- and NPPB-sensitive Cl^- current whose magnitude was inversely correlated with solution osmolarity (Figure 1A, inset). These data, coupled with those of Lyall *et al.* (Lyall *et al.*, 1999) that demonstrated effects of hyperosmotic stimuli on TRC gustatory responses, are consistent with the interpretation that the osmotic properties of taste stimuli may have profound effects on peripheral gustatory responses. The present study in particular demonstrates that increases in cell volume directly lead to activation of volume- or stretch-activated Cl^- channels that may contribute to the well-known gustatory response to water (Zotterman, 1956; Shingai, 1980).

Nature of the hypoosmotic response in taste receptor cells

Using whole-cell patch clamp recording, the perfusion of stimuli that varied only in their osmolarity led to significant changes in the current–voltage relationship generated in response to a voltage ramp from –90 to +60 mV. This hypoosmotic response was evident as an increase in the slope of the *I–V* curve (Figure 1A). The net response ($I_{HYPO\text{-}T}$) exhibited a moderate outwardly rectifying conductance (Figures 1A, 2 and 4). Though the ability to respond to changes in solution osmolarity is a feature of many cells types, not all the TRCs in the present study responded to decreases in osmolarity. Approximately one-third of TRCs could be classified as non-responsive; these cells showed neither the changes in conductance nor the change in membrane capacitance (surface area) that characterized the responsive TRCs. Our preliminary study that looked at the distribution of AQP-1, -2 and -5 in rat TRCs using antibodies directed against these water channels (Kim *et al.*, 1999) has shown that ~ 50% of TRCs contain these AQP molecules. Though in the present study I have not attempted to determine a correlation between AQP expression and the hypoosmotic responses, one explanation for the non-responsive TRCs may be that they lack a highly permeable route for water entry needed to mount a fast (<1 min) response to hypoosmotic stimuli.

The responses to hypoosmotic solutions were also characterized by marked adaptation despite the continued presence of the stimulus (Figure 1B). This adaptation was evident by both the adaptation of $I_{HYPO\text{-}T}$ and the change in membrane surface area. Adaptation of changes in membrane capacitance and $I_{HYPO\text{-}T}$ suggests that TRCs are capable of recovering from prolonged changes in the osmolarity of the extracellular milieu. A similar time course for adaptation of water responses in the frog (Andersson and Zotterman, 1950; Nomura and Ishizaki, 1972) and rat (Shingai, 1980) has been reported. Adaptation of hypoosmotic responses is common in a variety of cell types that function as osmometers (Bourque and Oliet, 1997; van der Wijk *et al.*, 2000) and it may be a mechanism to protect the cells from cytoskeletal damage during prolonged hypoosmotic conditions. This response, termed the regulatory volume decrease (RVD), is a typical feature of most cell types that display these types of swelling-activated Cl^- currents ($I_{Cl,swell}$) and follows a similar time course to that shown in Figure 1B [for a review see (Hume *et al.*, 2000)]. TRC responses to hyperosmotic solutions, on the other hand, did not show a similar type of adaptation (Lyall *et al.*, 1999).

Ion substitution experiments revealed that the outwardly rectifying $I_{HYPO\text{-}T}$ was a Cl^- current since changes in Cl^- concentration produced changes in the reversal potential for $I_{HYPO\text{-}T}$ that were closely predicted by the GHK equation (Figure 3). Because I could not be sure that the process of regulatory volume decrease did not begin before the maximal activation of $I_{HYPO\text{-}T}$, I have chosen to assume that the Cl^- concentration inside the cell did not appreciably change during osmotic-induced swelling. If the increase in surface area did lead to a significant reduction in intracellular Cl- concentration and I have overestimated $[Cl^-]_{in}$, then one would predict a slight (a few millivolts) shift in E_{Cl} toward a more negative value. Nonetheless, the relative permeability sequence for the various anions, which is an important defining characteristic of $I_{HYPO\text{-}T}$, would remain the same. I determined the permeability sequence for $I_{HYPO\text{-}T}$ in the present study was $SCN^- \geq I^- \geq Br^- > Cl^- \geq F^- \geq$ isethionate$^-$ > gluconate$^-$, which follows the relative permeability for the fairly ubiquitous $I_{Cl,swell}$ (Jentsch *et al.*, 1999). Similarities between $I_{HYPO\text{-}T}$ described in the present study and the well-described $I_{Cl,swell}$ extend into the pharmacology of this current. Both the stilbene derivative DIDS and the Cl^- channel inhibitor NPPB inhibited $I_{HYPO\text{-}T}$ in a manner consistent with these compounds' ability to inhibit $I_{Cl,swell}$ (Hume *et al.*, 2000). Thus, from all indications, the hypoosmotic-activated current in TRCs appears qualitatively similar to the $I_{Cl,swell}$ present in a variety of cell types.

The molecular identity of $I_{Cl,swell}$ remains unclear. There is controversy about which, if any, of the identified ClC gene family of Cl^- channels (Jentsch *et al.*, 1999) mediates the swelling-activated conductance. One of the potential

candidates for $I_{Cl,swell}$ remains ClC-3. When expressed, this current shows outward rectification, an $I^- > Cl^- > F^-$ permeability sequence and sensitivity to stilbene derivatives (Duan *et al*., 1997; Shimada *et al*., 2000). However, experiments using heterologous expression of ClC-3 are complicated first, by the fact that this channel is expressed to some degree in most cells types, including those used for heterologous expression, such as *Xenopus* oocytes, and Chinese hamster ovary and NIH/3T3 cell lines (Li *et al*., 2000; Stobrawa *et al*., 2001); and second by the fact that other laboratories have been unable to reproduce these expression data (Li *et al*., 2000). Further support for the involvement of ClC-3 in $I_{Cl,swell}$ has come from a recent study by Duan and colleagues (Duan *et al*., 2001) that used antibodies against ClC-3 to inhibit the native $I_{Cl,swell}$ in guinea pig cardiac myocytes and in heterologously expressed ClC-3 in *Xenopus* oocytes, suggesting that ClC-3 was an important contributor to this current. Despite this evidence, ClC-3 knockouts exhibit normal $I_{Cl,swell}$ responses in both hepatocytes and pancreatic acinar cells, two cells that express substantial $I_{Cl,swell}$ in wild-type animals (Stobrawa *et al*., 2001). This study concluded that ClC-3 is an intracellular channel similar to the closely related ClC-4 and -5 channels, whose properties vary dramatically from the native $I_{Cl,swell}$ (Friedrich *et al*., 1999). Clearly, the molecular nature of the channel mediating $I_{Cl,swell}$ and $I_{HYPO\text{-}T}$ remains an open question (Clapham, 2001).

At least one member of the ClC family of chloride channels has been identified in taste receptor cells. Despite the fact that ClC-3 remains the only viable candidate of the ClC family for mediating $I_{Cl,swell}$, it is not apparently found in mammalian TRCs. Miyamoto and colleagues (Miyamoto *et al*., 2001) failed to identify ClC-3 protein using immunocytochemical techniques, but did locate ClC-2 protein on the basolateral membranes of both taste cells and epithelial cells in the lingual epithelium of C57BL/6 mice. They suggested that ClC-2 channels might contribute to the inwardly rectifying current mediating KCl responses in TRCs. ClC-2, however, is unlikely to be involved in generating the $I_{HYPO\text{-}T}$ reported in the present study. ClC-2 currents are inwardly rectifying, have a $Cl^- > Br^- > I^-$ permeability sequence, and are inhibited by millimolar concentrations of Cd^{2+} (Okada *et al*., 1998; Wills and Fong, 2001), features distinct from $I_{HYPO\text{-}T}$ in rat TRCs.

Implications of $I_{HYPO\text{-}T}$ in taste receptor cells

The identification of an $I_{Cl,swell}$-like conductance in rat taste receptor cells, which I have termed $I_{HYPO\text{-}T}$ to represent the hypoosmotic-activated current in taste cells, implies that mammalian TRCs are capable of responding electrophysiologically to decreases in extracellular solution tonicity. In addition, the changes in both membrane capacitance (surface area) (Thiele *et al*., 1998) and $I_{HYPO\text{-}T}$ returned to near control levels despite continual perfusion of the hypoosmotic stimulus. This result is consistent with the interpretation that TRCs are capable of RVD. Though in taste cells the mechanism of RVD has not been investigated, it is likely to involve the efflux of solutes during hypoosmotic stimulation, as is the case in other cell types (Bond *et al*., 1998; Deleuze *et al*., 1998; Song *et al*., 1998).

In the present study, stimuli were applied to the entire surface of the taste cells and not restricted to their apical membranes, as is the case *in vivo*. As such, no conclusions can easily be drawn about whether these responses reflect those that are attributable to taste responses or merely compensatory responses during osmotic changes as might occur in the interstitial fluid surrounding the taste bud. Nonetheless, there are several parallels between this and other studies that are suggestive of the fact that this conductance may also be important for gustatory processing. First, previous work from my laboratory has shown that TRCs from the fungiform papillae contain apically localized AQP-5 water channels (Kim *et al*., 1999) that would provide a route for rapid water entry during such hypoosmotic stimulation. Thus, irrespective of the cellular localization of these putative stretch-activated channels that underlie $I_{HYPO\text{-}T}$, water entry through these apical channels could lead to activation of this Cl^- conductance.

Secondly, in many cell types there exists a basally active Cl^- conductance that has all the hallmarks of both $I_{HYPO\text{-}T}$ and $I_{Cl,swell}$, including enhancement by swelling, outward rectification, $I^- > Br^- > Cl^-$ permeability sequence and stilbene derivative sensitivity (Duan and Nattel, 1994; Duan *et al*., 1997). It has been hypothesized that this current, termed $I_{Cl,b}$, is actually the same current as $I_{Cl,swell}$ (Hume *et al*., 2000), and that this conductance may be partially active under normal, isotonic conditions. Interestingly, a recent report implicated a NPPB-sensitive Cl^- conductance in the transduction of sour tastants (Miyamoto *et al*., 1998). This conductance was inhibited by NPPB applied basolaterally in the same concentration range (Miyamoto *et al*., 1998) as the NPPB block of $I_{HYPO\text{-}T}$ (Figure 5), and, like $I_{HYPO\text{-}T}$, was outwardly rectifying and Gd^{3+}-insensitive. Thus, if $I_{HYPO\text{-}T}$ represents a constitutively active current in TRCs, then this may be one of the targets for modulation by sour tastants (e.g. acids). Additionally, $I_{Cl,b}$ has been shown to be inhibited by hyperosmotic stimuli (Duan *et al*., 1995), suggesting that it may contribute not only to hypoosmotic responses, but also to hyperosmotic responses, and may, in this way, contribute to the hyperosmotic responses reported by Lyall *et al*. (Lyall *et al*., 1999). Further studies aimed at elucidating the contribution of $I_{HYPO\text{-}T}$ to sour taste transduction and hyperosmotic taste responses appear warranted.

A third potential role of this conductance in gustatory processing may be in its contribution to 'water' taste (Zotterman, 1956; Storey and Johnson, 1975; Shingai, 1980). The gustatory response to water has been demonstrated in a variety of species [for a review see (Lindemann, 1996)]. In cat (Cohen *et al*., 1955), pig (Zotterman, 1956) and frog (Andersson and Zotterman, 1950) tongue, afferent

nerve fibers were shown to respond to orally applied hypoosmotic (low ionic strength) stimuli by increasing their firing rates. These responses were sensitive to mucosal Cl^- concentrations, suggesting that this anion played a role in the water response. This dependence of the water response on mucosal Cl^- was supported by more recent studies in the frog tongue that demonstrated that increasing the mucosal Cl^- inhibited the water response (Okada *et al*., 1993). If $I_{HYPO\text{-}T}$ is involved in this response, increasing mucosal (extracellular) Cl^- concentrations would be predicted to reduce net efflux of Cl^- and hence decrease the net inward current, provided that these channels could sense mucosal ion concentrations (i.e. were apically localized). Moreover, the water response in the frog was sensitive to stilbene derivatives like DIDS (Okada *et al*., 1993). In the rabbit, water responses were similarly inhibited by extracellular Br^-, I^- and Cl^- ions (Shingai, 1977), all of which were markedly permeant through the hypoosmotic-activated channels in the present study. Taken together, there is a good correlation between the properties known for the water response in taste cells and the properties of $I_{HYPO\text{-}T}$ described in the current study.

An inherent property of the water response in the oral cavity is that the most vigorous responses occur in the laryngeal water receptors and, by comparison, water responses originating in the lingual taste buds are quite modest (Shingai and Shimada, 1976; Smith and Hanimori, 1991). I have shown that all the taste receptor cells investigated in this study respond in a similar fashion to hypoosmotic stimuli (Figure 2), suggesting a common mechanism underlying the generation of $I_{HYPO\text{-}T}$. Though a slightly greater percentage of hypoosmotic-responsive cells was found in the area of the epiglottis (85.7% of cells responded versus 61.1% in the fungiform taste buds), there was no significant difference in the density of $I_{HYPO\text{-}T}$ in various areas of the oral cavity (Figure 2F), arguing against higher levels of expression of hypoosmotic-activated channels mediating the differences in water responsiveness between the tongue and the back of the oral cavity. It is possible that local differences in ion concentrations, either intracellularly or extracellularly, or differences in cellular expression (apical versus basolateral) of the channels underlying $I_{HYPO\text{-}T}$ may play a role in generating this difference.

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References

Andersson, B. and **Zotterman, Y.** (1950) *The water taste in the frog.* Acta Physiol. Scand., 20, 95–100.

Béhé, P., DeSimone, J.A., Avenet, P. and **Lindemann, B.** (1990) *Membrane currents in taste cells of the rat fungiform papillae: evidence for two types of Ca currents and inhibition of K currents by saccharin.* J. Gen. Physiol., 96, 1061–1084.

Bond, T.D., Ambikapathy, S., Mohammad, S. and **Valverde, M.A.** (1998) *Osmosensitive Cl^- currents and their relevance to regulatory volume decrease in human intestinal T_{84} cells: outwardly vs. inwardly rectifying currents.* J. Physiol., 511, 45–54.

Bourque, C.W. and **Oliet, S.H.** (1997) *Osmoreceptors in the central nervous system.* Annu. Rev. Physiol., 59, 601–619.

Clapham, D.E. (2001) *How to lose your hippocampus by working on chloride channels.* Neuron, 29, 1–3.

Cohen, M.J., Hagiwara, S. and **Zotterman, Y.** (1955) *The response spectrum of taste fibres in the cat: a single fibre analysis.* Acta Physiol. Scand., 33, 316–332.

Cunningham, J.T., Wachtel, R.E. and **Abboud, F.M.** (1995) *Mechanosensitive currents in putative aortic baroreceptor neurons* in vitro. J. Neurophysiol., 73, 2094–2098.

Deleuze, C., Duvoid, A. and **Hussy, N.** (1998) *Properties and glial origin of osmotic-dependent release of taurine from the rat supraoptic nucleus.* J. Physiol., 507, 463–471.

Doolin, R.E., and **Gilbertson, T.A.** (1996) *Distribution and characterization of functional amiloride sensitive sodium channels in rat tongue.* J. Gen. Physiol., 107, 545–554.

Duan, D. and **Nattel, S.** (1994) *Properties of single outwardly rectifying Cl^- channels in heart.* Circ. Res., 75, 789–795.

Duan, D., Fermini, B. and **Nattel, S.** (1995) *Alpha-adrenergic control of volume-regulated Cl^- channels in rabbit atrial myocytes. Characterization of a novel ionic regulatory mechanism*. Circ. Res., 77, 379–393.

Duan, D., Winter, C., Cowley, S., Hume, J.R. and **Horowitz, B.** (1997) *Molecular identification of a volume-regulated chloride channel.* Nature, 390, 417–421.

Duan, D., Zhong, J., Hermoso, M., Satterwhite, C.M., Rossow, C.F., Hatton, W.J., Yamboliev, I., Horowitz, B. and **Hume, J.R.** (2001) *Functional inhibition of native volume-sensitive outwardly rectifying anion channels in muscle cells and* Xenopus *oocytes by anti-ClC3 antibody*. J. Physiol., 531, 437–444.

Echevarría, M. and **Ilundáin, A.A.** (1998) *Aquaporins.* J. Physiol. Biochem., 54, 107–118.

Feldman, M. and **Barnett, C.** (1995) *Relationships between the acidity and osmolality of popular beverages and reported postprandial heartburn.* Gastroenterology, 108, 125–131.

Friedrich, T., Breiderhoff, T. and **Jentsch, T.J.** (1999) *Mutational analysis demonstrates the ClC-4 and ClC-5 directly mediate plasma membrane currents.* J. Biol. Chem., 274, 896–902.

Gilbertson, T.A. (1998) *Gustatory mechanisms for the detection of fat.* Curr. Opin. Neurobiol., 8, 447–452.

Gilbertson, T.A. and **Fontenot, D.T.** (1998) *Distribution of amiloride-sensitive sodium channels in the oral cavity of the hamster.* Chem. Senses, 23, 495–499.

Gilbertson, T.A. and **Kinnamon, S.C.** (1996) *Making sense of chemicals.* Chem. Biol., 3, 233–237.

Gilbertson, T.A., Roper, S.D. and **Kinnamon, S.C.** (1993) *Proton currents through amiloride-sensitive Na^+ channels in isolated hamster taste cells: enhancement by vasopressin and cAMP.* Neuron, 10, 931–942.

Gilbertson, D.M., Monroe, W.T., Milliet, J.R., Caprio, J. and **Gilbertson, T.A.** (1997a) *Citrate ions enhance behavioral and cellular responses to taste stimuli.* Physiol. Behav., 62, 491–500.

Gilbertson, T.A., Fontenot, D.T., Zhang, H., Liu, L. and **Monroe, W.T.** (1997b) *Fatty acid modulation of K^+ channels in taste receptor cells: gustatory cues for dietary fat.* Am. J. Physiol., 272, C1203–C1210.

Gilbertson T.A., Siears, N.D., Mercante, J.W., Zhang, H. and **Kim, I.** (2000) *Hypoosmotic stimuli activate a chloride conductance in taste cells.* Chem. Senses, 25, 687.

Hall, S.K., Zhang, J. and **Lieberman, M.** (1997) *An early transient current is associated with hyposmotic swelling and volume regulation in embryonic chick cardiac myocytes.* Exp. Physiol., 82, 43–54.

Heymann, J.B. and **Engel, A.** (1999) *Aquaporins: phylogeny, structure, and physiology of water channels.* News Physiol. Sci., 14, 187–193.

Hume, J.R., Duan, D., Collier, M.L., Yamazaki, J. and **Horowitz, B.** (2000) *Anion transport in the heart*. Physiol. Rev., 80, 31–81.

Ishiko, N. and **Sato, Y.** (1973) *Gustatory coding in the cat chorda tympani fibers sensitive and insensitive to water.* Jpn. J. Physiol., 23, 275–290.

Jentsch, T.J., Friedrich, T., Schriever, A. and **Yamada, H.** (1999) *The CLC chloride channel family.* Pflügers Arch. Eur. J. Physiol., 437, 783–795.

Kim I., Liu, L., Siears, N.L., Kim, J.H. and **Gilbertson, T.A.** (1999) *Regionalized expression of aquaporins suggests a role for water channels in osmotic sensing by taste cells.* Soc. Neurosci. Abstr., 25, 2184.

Köhler, R., Distler, A. and **Hoyer, J.** (1998) *Pressure-activated cation channel in intact rat endocardial endothelium.* Cardiovasc. Res., 38, 433–440.

Law, R.O. (1996) *Volume regulation and the efflux of amino acids from cells in incubated slices of rat cerebral cortex. II. Dependence on Ca^{2+} ions.* Biochim. Biophys. Acta, 1314, 43–48.

Li, X., Shimada, K., Showalter, L.A. and **Weinman, S.A.** (2000) *Biophysical properties of ClC-3 differentiate it from swelling-activated chloride channels in Chinese hamster ovary-K1 cells.* J. Biol. Chem., 275, 35994–35998.

Lindemann, B. (1996) *Taste reception.* Physiol. Rev., 76, 719–766.

Lyubartsev, A.P. and **Laaksonen, A.** (1997) *Osmotic and activity coefficients from effective potentials for hydrated ions.* Physical Rev. E, 55, 5689–5696.

Lyall, V., Heck, G.L., DeSimone, J.A. and **Feldman, G.M.** (1999) *Effects of osmolarity on taste receptor cell size and function.* Am. J. Physiol., 277, C800–C813.

Miyamoto, T., Fujiyama, R., Okada, Y. and **Sato, T.** (1998) *Sour taste transduction involves activation of an NPPB-sensitive conductance in mouse taste cells.* J. Neurophysiol., 80, 1852–1859.

Miyamoto, T., Miyazaki, T., Fujiyama, R., Okada, Y. and **Sato, T.** (2001) *Differential transduction mechanisms underlying NaCl- and KCl-induced responses in mouse taste cells.* Chem. Senses, 26, 67–77.

Morris, C.E. (1990) *Mechanosensitive ion channels.* J. Membr. Biol., 113, 93–107.

Myers, W.E., Hettinger, T.P. and **Frank, M.E.** (1993) *Inhibition of hamster chorda tympani neural response by copper chloride.* Chem. Senses, 18, 495–507.

Ninomiya, Y., Kurenuma, S., Nomura, T., Uebayashi, H. and **Kawamura, H.** (1992) *Taste synergism between monosodium glutamate and 5′-ribonucleotide in mice.* Comp. Biochem. Physiol. A, 101, 97–102.

Nomura, H., and **Ishizaki, M.** (1972) *Stimulation mechanism of the water response in the frog: roles of anions in the activity of a chemoreceptor.* Bull. Tokyo Dent. Coll., 13, 21–52.

Okada, Y., Miyamoto, T. and **Sato, T.** (1993) *The ionic basis of the receptor potential of frog taste cells induced by water stimuli.* J. Exp. Biol., 174, 1–17.

Okada, Y., Oiki, S., Hazama, A. and **Morishima, S.** (1998) *Criteria for the molecular identification of the volume-sensitive outwardly rectifying Cl^- channel.* J. Gen. Physiol., 112, 365–367.

Pérez-Samartín, A.L., Miledi, R. and **Arellano, R.O.** (2000) *Activation of volume-regulated Cl^- channels by ACh and ATP in* Xenopus *follicles.* J. Physiol., 525, 721–734.

Quasthoff, S. (1994) *A mechanosensitive K^+ channel with fast-gating kinetics on human axons blocked by gadolinium ions*. Neurosci. Lett., 169, 39–42.

Robinson, R.A., and **Stokes, R.H.** (1955) Electrolyte Solutions. Academic Press, New York.

Sato, T., Okada, Y. and **Miyamoto, T.** (1995) *Molecular mechanisms of gustatory transduction in frog taste cells*. Prog. Neurobiol., 46, 239–287.

Schiffman, S.S., Suggs, M.S., Sostman, A.L. and **Simon, S.A.** (1992) *Chorda tympani and lingual nerve responses to astringent compounds in rodents.* Physiol. Behav., 51, 55–63.

Schiffman, S.S., Suggs, M.S., Losee, M.L., Gatlin, L.A., Stagner, W.C. and **Bell, R.M.** (1995) *Effect of lipid-derived second messengers on electrophysiological taste responses in the gerbil.* Pharmacol. Biochem. Behav., 52, 49–58.

Shimada, K., Li, X., Xu, G., Nowak, D.E., Showalter, L.A. and **Weinman, S.A.** (2000) *Expression and canalicular localization of two isoforms of the ClC-3 chloride channel from rat hepatocytes.* Am. J. Physiol., 279, G268–G276.

Shingai, T. (1977) *Ionic mechanism of water responses in the laryngeal mucosa of the rabbit.* Jpn. J. Physiol., 27, 27–42.

Shingai, T. (1980) *Water fibers in the superior laryngeal nerve of the rat.* Jpn. J. Physiol., 30, 305–307.

Shingai, T. and **Shimada, K.** (1976) *Reflex swallowing elicited by water and chemical substances applied in the oral cavity, pharynx, and larynx of the rabbit.* Jpn. J. Physiol., 26, 455–469.

Singh, S.B., Sharma, A., Yadav, D.K., Verma, S.S., Srivastava, D.N., Sharma, K.N., and **Selvamurthy, W.** (1997) *High altitude effects on human taste intensity.* Aviat. Space Environ. Med., 68, 1123–1128.

Smith, D.V., and **Hanimori, T.** (1991) *Organization of gustatory sensitivities in hamster superior laryngeal nerve fibers.* J. Neurophysiol., 65, 1098–114.

Song, D., O'Regan, M.H. and **Phillis, J.W.** (1998) *Amino acid release during volume regulation by cardiac cells: cellular mechanisms.* Eur. J. Pharmacol., 341, 273–280.

Storey, A.T., and **Johnson, P.** (1975) *Laryngeal water receptors initiating apnea in the lamb.* Exp. Neurol., 47, 42–55.

Stobrawa, S.M., Breiderhoff, T., Takamori, S., Engel, D., Schweizer, M., Zdebik, A.A., Bösl, M.R., Ruether, K., Jahn, H., Draguhn, A., Jahn, R. and **Jentsch, T.J.** (2001) *Disruption of ClC-3, a chloride channel expressed on synaptic vesicles, leads to a loss of the hippocampus.* Neuron, 29, 185–196.

Thiele, I., Warth, R., Bleich, M., Waldegger, S., Lang, F. and **Greger, R.** (1998) *Osmotically induced conductance and capacitance changes in* in vitro *perfused rectal gland tubules of* Squalus acanthias. Kidney Blood Press. Res., 21, 317–324.

van der Wijk, T., Tomassen, S.F., de Jonge, H.R. and **Tilly, B.C.** (2000) *Signalling mechanisms involved in volume regulation of intestinal epithelial cells.* Cell. Physiol. Biochem., 10, 289–96.

Wenning, A. (1999) *Sensing effectors make sense.* Trends Neurosci., 22, 550–555.

Wills, N.K. and **Fong, P.** (2001) *ClC chloride channels in epithelia: recent progress and remaining puzzles.* News Physiol. Sci., 16, 161–166.

Xu, W.X., Kim, S.J., So, I., Kang, T.M., Rhee, J.C. and **Kim, K.W.** (1997) *Volume-sensitive chloride current activated by hyposmotic swelling in antral gastric myocytes of the guinea pig.* Pflügers Arch. Eur. J. Physiol., 435, 9–19.

Zotterman, Y. (1956) *Species differences in water taste.* Acta Physiol. Scand., 37, 60–70.

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