Characterization of Chloride Currents and Their Noradrenergic Modulation in Rat Taste Receptor Cells

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Herness, M. Scott and Xiao-Dong Sun. Characterization of chloride currents and their noradrenergic modulation in rat taste receptor cells. J. Neurophysiol. 82: 260–271, 1999. Taste receptor cells contain a heterogeneous array of voltage-dependent ion conductances that are essential components for the transduction of gustatory stimuli. Although mechanistic roles have been proposed for several cationic conductances, the understanding of anionic currents is rudimentary. This study characterizes biophysical and pharmacological properties of chloride currents in rat posterior taste cells using whole cell patch-clamp recording technique. Taste cells express a heterogeneous array of chloride currents that displayed strong outward rectification, contained both calcium–dependent and calcium–independent components, and achieved a maximal conductance of almost 1 nS. Reversal potentials altered predictably with changes in chloride concentration. Currents were sensitive to inhibition by the chloride channel pharmacological agents DIDS, SITS, and niflumic acid but were insensitive to 9-AC. Adrenergic enhancement of chloride currents, present in other cell types, was tested on taste cells with the β-adrenergic agonist isoproterenol (ISP). ISP enhanced the outwardly rectifying portion of the chloride current. This enhancement was calcium dependent and was blocked by the β-adrenergic antagonist propranolol. Collectively these observations suggest that chloride currents may participate not only in usually ascribed functions such as stabilization of the membrane potential and volume regulation but additionally play active modulatory roles in the transduction of gustatory stimuli.

INTRODUCTION

Taste receptor cells are differentiated epithelial cells that detect the presence of sapid stimuli within the oral cavity and relay this information to the CNS via sensory afferent nerves. Taste cells are electrically excitable and utilize a diverse array of voltage-gated ion channels to elicit either receptor potentials or action potentials in response to appropriate stimulation. The detailed characterization of these ion channels is prerequisite to elucidating the variety of transduction mechanisms employed by these cells. Many of these currents have been fully or partially characterized. In the mammalian taste receptor cell, these include voltage-dependent sodium channels (Doolin and Gilbertson 1996; Herness and Sun 1995), delayed-rectifier, transient, and calcium-activated outward potassium currents (Béhé et al. 1990; Chen et al. 1996), inwardly rectifying potassium currents (Sun and Herness 1996b), and calcium currents (Béhé et al. 1990; Chen and Herness 1997). In rat posterior taste cells, these channels display an obvious diversity in expression across cells. For example, many taste cells do not express voltage-dependent sodium channels and are hence incapable of eliciting action potentials. Potassium channel expression is also very heterogeneous and strongly correlated with action potential duration. The differences in distribution of ion channels parallel well-documented differences in chemosensitivity that also occurs across taste cells. In studies of taste cells, one major class of channel, chloride channels, has yet to be characterized.

Membrane conductance to chloride, once dismissed as artifact and generically termed “leak,” is now recognized to participate in a variety of physiological roles that include stabilization of the membrane potential, volume regulation, and salt transport. These diverse physiological roles are matched by the broad heterogeneity of this channel type. Presently, at least nine genes are recognized that encode chloride channel proteins (CLC) that function as either chloride channels or putative chloride channels (Jentsch 1996). There are several hypothetical reasons to suggest possible roles of chloride currents in the normal physiology of taste cells. First, fluid and electrolyte secretion is known to be coupled to vectorial chloride movement (for reviews see Anderson et al. 1992; Frizzell and Morris 1994) and because taste cells are implicated in salt transport across the tongue (DeSimone et al. 1981, 1984; Mierson et al. 1994), chloride channels are likely to be involved. Second, a major chloride conductance, cystic fibrosis transmembrane conductance regulator (CFTR), is cAMP dependent (e.g., Gadsby et al. 1995) and cAMP is an important intracellular messenger in posterior taste receptor cells (Herness et al. 1997). Third, chloride channels play essential roles in regulatory volume decrease produced by changes in osmolarity (Niell et al. 1996), and taste cells are routinely subjected to osmotic extremes from water to hyperosmotic foodstuffs. Finally, chloride currents are sometimes active participants in electrical responses of excitable cells (e.g., Harvey 1996) and hence may play yet unrecognized roles in gustatory transduction.

Some preliminary characterizations of chloride currents in amphibians taste cells have been reported. A calcium-dependent outward chloride current has been reported in Necturus taste cells (MCRM and Roper 1991). With the use of intracellular techniques, it was observed that calcium influx during the action potential was sufficient to stimulate a calcium-dependent chloride current. These channels appear to be on both apical and basolateral membranes. Their function was postulated as twofold, to help discriminate chloride and nonchloride salts, and to help shape the receptor potential responses initiated by taste stimuli. It was later reported (Taylor...
and Roper 1994) with patch-clamp techniques, that this current is sensitive to SITS and DIDS and that it may play a role in adaptation, by terminating a long depolarizing receptor potential.

The present study was designed to investigate the nature of the chloride conductance in mammalian taste receptor cells using the whole cell configuration of the patch-clamp recording technique. Biophysical and pharmacological evidence is presented that taste receptor cells likely possess multiple chloride conductances.

**Methods**

All experiments were performed on isolated taste receptor cells dissociated from circumvallate and foliate papillae of the rat tongue using standard patch-clamp procedures in the whole cell recording mode.

**Dissociation procedure**

Taste receptor cells were dissociated from excised regions of the posterior rat tongue as previously described (Herness 1989). Lingual tissue was excised from an animal that had previously reached a surgical level of anesthesia with an intramuscular injection of 0.09 ml/100 gm body wt Ketamine/Acepromazine mixture (91 mg/ml Ketamine, Fort Dodge Laboratories: 0.09 mg/ml Acepromazine, Butler Laboratories). Papillae were blocked from tongue tissue and incubated in a cysteine-activated (1 mg/ml Papain/divalent–free bicarbonate–buffered solution (14 U/ml) for several hours at 32°C in 5% CO₂–95% air. Cells were dissociated in a pseudo-extracellular fluid (ECF) by mild agitation. Some papillae were maintained in ice-cold ECF solution for later dissociation. Dissociated taste receptor cells were easily identified by their characteristic morphology. However, in the dissociated state it is not always possible to distinguish apical from basolateral domains.

**Solutions**

The divalent-free solution for enzymatic incubation was composed of (in mM) 65 NaCl, 20 KCl, 26 NaHCO₃, 2.5 NaH₂PO₄ • H₂O, 20 d-glucose, and 1 EDTA. The standard ECF solution used for the dissociation procedure included (in mM) 126 NaCl, 1.25 NaH₂PO₄ • H₂O, 5 KCl, 5 NaHEPES, 2 MgCl₂, 2 CaCl₂, and 10 glucose; it was pH adjusted to 7.4 with NaOH.

The composition of intracellular fluid (ICF) used for for filling the recording pipette consisted of (in mM) 24 CsCl, 116 methanesulfonic acid (cesium salt), 2 MgCl₂, 1 CaCl₂, 11 EGTA, 5 HEPES, and 4 ATP (magnesium salt); this solution was adjusted to a final pH of 7.2 with CsOH and yielded a final chloride concentration of 30 mM. In some cases, a high chloride ICF was used with a final chloride concentration of 146 mM; its composition was identical to the previous ICF except that the CsCl concentration was 140 mM and methanesulfonic acid was omitted. A potassium-free extracellular solution was employed for the bath solution; it consisted of (in mM) 126 NaCl, 5 HEPES, 2 CaCl₂, 2 MgCl₂, and 10 glucose with a final chloride concentration of 134 mM. For some experiments, this solution may have additionally contained 1 BaCl₂ and/or 0.5 or 1 CdCl₂ to inhibit either inwardly rectifying potassium currents or calcium currents, respectively. The extracellular recording solution was pH adjusted to 7.4 with Tris base.

For ion substitution experiments, where either intracellular or extracellular chloride concentration was manipulated, chloride was substituted with the cesium salt of methanesulfonic acid for ICF solutions or the sodium salt of isethionic acid for ECF solutions. For pharmacological analysis of chloride currents, agents were dissolved in the superfusing solution. These included two stilbene derivatives, 4,4’-disothiocyanostilbene-2,2’-disulfonic acid (DIDS, Sigma) and 4-aceatamido-4’-isothiocyanostilbene-2,2’-disulidonic acid (SITS, Sigma), each at 500 μM, niflumic acid (Sigma) tested at 500 or 1,000 μM, or 9-anthracene carboxylic acid (9-AC, Sigma) at 500 μM.

**Whole cell electrophysiological recording**

Micropipettes were pulled on a gas-cooled multistage puller from 1.5-mm OD borosilicate glass (World Precision Instruments, Sarasota, FL) and were fire polished. Resistances were typically 2–4 MΩ when filled with ICF and measured in ECF. Junction potentials were corrected before the electrode contacted the cell. [Although small junction potentials are introduced when using silver:silver chloride electrodes during changes of chloride concentration, an agar bridge was not employed during experiments with altered extracellular chloride (Figs. 2 and 7) because these measurements were largely qualitative and agar bridge are, at best, imperfect solutions (Neher 1992). Nevertheless, artifacts of several millivolts may have been introduced during the experiments with altered chloride concentrations for this reason.] The pipette tip was positioned to contact the cell membrane, and negative pressure was applied to its interior to facilitate gigaseal formation. Seal resistances were on the order of several decades of gigahoms. Further negative pressure was applied to enter whole cell recording mode.

Fast and slow capacitance compensation was employed as necessary with amplifier controls. Cell membrane capacitance and uncompensated series resistance were adjusted to produce optimal transient balancing. Membrane capacitance was 3–6 pF; series resistance averaged 10 MΩ. Low-pass filtering due to resistance-capacitance coupling was considered minimal. The product of these factors produces a time constant of 30–60 μs or a cutoff frequency (1/2πRC) of 2.6–5.3 kHz.

Data were acquired with a high-impedance amplifier and a high-resistance feedback headstage (Axopatch 200A; Axon Instruments), a 486 computer equipped with a 12-bit 330-KHz A/D converter (Digidata 1200; Axon Instruments), and a commercially available software program (pCLAMP, version 6.0.3; Axon Instruments). Membrane currents were acquired after low-pass filtering with a cutoff frequency of 5 kHz (at ~3 dB). A software-driven D/A converter generated the voltage protocols. In some situations, currents were measured with voltage protocols using standard step potentials with a holding potential of 0 mV and a series of 80-ms command potentials applied in 20-mV increments from final potentials ranging −140 to +120 mV and acquired with a sampling rate of 195 μs. Most data were acquired with the use of a ramp protocol that clamped the membrane potential from −130 to +120 mV over a period of 3 s (83.3 mV/s) using a sampling rate of 2 ms. Leak subtraction was not employed. Experiments with a P4/leak subtraction protocol resulted in current profile that was outwardly rectifying; however, much of the linear component was removed. Because this linear portion of the total current is present when chloride is the major conducting ion and is inhibited by chloride channel blockers, the leak subtraction protocol was concluded to inappropriately remove a linear component of the total chloride current. Moreover, in addition to confounding current-voltage (I–V) relationships, leak subtraction would complicate interpretations of zero-current potentials and steady-state current amplitudes.

In general, obvious rundown of currents was not observed. The findings that chloride currents responded appropriately to manipulations of the bath solutions during the recording session, including altering chloride concentrations, altering calcium currents, and combinations of isopropenol followed by a second manipulation (either low chloride, cadmium, or propanolol), collectively argue that current was behaving as expected during the recording session without obvious signs of rundown.

Recordings were made at room temperature. Positive currents reflect the inward flux of chloride; negative currents represent an outward chloride flux.
Data were analyzed with a combination of off-line software programs that included a software acquisition suite (pCLAMP, Axon Instruments) and a technical graphics/analysis program (Origin; MicroCal Software).

Theoretical equilibrium potentials were calculated according to the Nernst equation using chloride concentrations of intracellular and extracellular solutions. Whole cell chloride conductance was calculated as

\[ g_{Cl} = \frac{i}{E_m - E_{rev}} \]

where \( E_m \) is the membrane voltage and \( E_{rev} \) is the reversal potential. Conductance voltage relationships were fit with a Boltzmann distribution

\[ G_{Cl} = \frac{G_{max}}{1 + \exp(E_{1/2}/\alpha_g)} \]

where \( G_{max} \) is the maximal conductance, \( E_{1/2} \) is the membrane potential at which conductance was 50% of its maximal value, and \( \alpha_g \) is the steepness coefficient that corresponds to \( kT/2e \) (where \( k \) is Boltzmann’s constant, \( T \) is the absolute temperature, \( \epsilon \) is the equivalent charge of the gating particle, and \( e \) is the elementary charge). Pooled one-tailed Student’s \( t \)-test was used to evaluate the statistical significance of the difference between means. Values of \( P < 0.05 \) were considered to indicate statistical significance. Data are presented as means ± SE.

RESULTS

Chloride currents were recorded from all cells that previously satisfied criteria for whole cell recording. These currents were considered to be conducted by chloride ions because they were dependent on the presence of chloride in either the bathing or pipette solutions, the currents reversed at a predicted Nernstian potential, and they demonstrated a pharmacology consistent with that of chloride channels.

Isolation of chloride currents

A series of inward and outward currents was recorded from dissociated taste receptor cells in response to hyperpolarizing and depolarizing voltage steps delivered from a holding potential of 0 mV. Currents were recorded in potassium-free ECF and ICF solutions that established a chloride gradient of 134/30 mM (outside/inside). Data from a representative cell are presented in Fig. 1A: the I-V plot for this cell, obtained from steady-state values, is illustrated in Fig. 1B. Evident in both the current traces and the I-V plot is a prominent outward rectification with little time dependence to the current over the 80-ms pulse. (We use the standard convention of referring to positive currents as outward currents, although they literally represent an inward movement of chloride ions.) Additionally, these currents often produced a distinctive tail current at the break of either large hyperpolarizing pulses (greater than −80 mV) or large depolarizing pulses (>70 mV). Tail currents appear to be complex, receiving contributions from a variety of voltage-dependent conductances; they will not be further considered in this communication. Currents reversed at a predicted Nernstian reversal potential for the chloride gradient used for these recording, between −35 and −40 mV. The predicted potential for a chloride gradient of 134 mM external and 30 mM internal chloride is −38 mV.

A ramp command potential was also employed to evoke membrane currents. Data from 12 cells, using a ramp protocol, are presented in Fig. 1C. Current was ramped from −130 to 120 mV from a holding potential of 0 mV (inset of Fig. 1C) using a rate \( (dV/dt) \) of 83 mV/s. The ramp potential facilitated data acquisition by evoking a broad membrane range over a short period of time. Additionally, the combined use of a 0-mV holding potential, the ionic composition of the recording solutions, and more gradual change of the clamped membrane potential (compared with abrupt step changes) helped to ensure inactivation of other membrane conductances. This protocol

![Fig. 1](http://jn.physiology.org/)

Whole cell chloride currents recorded from dissociated rat posterior taste receptor cells. A: representative whole cell chloride currents from a single cell evoked using standard step command potentials from a holding potential of 0 mV. Currents were outwardly rectifying. At the break of depolarizing step potentials, tail currents were evident. The voltage protocol used to elicit these currents is illustrated at top. B: current-voltage plot for the current records presented in (A) showing outward rectification and a predicted reversal potential of about −35 mV. C: chloride currents recorded using a ramp potential (illustrated in inset). Data are means of 12 cells. The current is outwardly rectifying and reverses close to −30 mV. The dotted line is a linear regression performed on the outward portion of the current \( (r = 0.9967) \) that yielded a slope conductance of 0.82 nS. D: chord conductance of the mean current presented in C. Data were fit with a Boltzman relationship that yielded a half-maximal voltage of about −3.5 mV and a steepness coefficient of 21 mV.
was used for all subsequent experiments. As with currents evoked by step potentials, the data displayed pronounced outward rectification and reversed at the appropriate membrane potential. The dotted line represents extrapolated data using the linear portion of the outward current. The linear regression of this area of the I-V curve produced a slope conductance of 0.82 nS ($r = 0.9997$). Chord conductance (Fig. 1D) plateaued at 0.7 nS and was adequately described by a Boltzmann relationship with a half-maximal potential of −3.5 mV and a steepness coefficient of 21 mV per e-fold. The steepness coefficient produced an equivalent gating charge of 1.2. This value agrees quite well with the typically modest voltage dependence of the equivalent gating charge of 1.2–2.0 that are ascribed to chloride channels (Hille 1992). As a comparison with other membrane conductances in these cells, we have previously measured in posterior taste receptor cells a voltage-dependent sodium conductance of 7.3 nS (Herness and Sun 1995) and total outward potassium conductance of 23 nS (Chen et al. 1996). Although little current is evoked at membrane potentials that correspond to the resting potential in these cells, a measurable conductance does occur. When considered with the high-input resistant of these cells (5–20 GΩ), it is likely that these currents can contribute to total membrane conductance at zero-current potentials.

That currents isolated under these conditions were in fact conducted by chloride was confirmed by ionic substitution of either external or internal chloride concentrations. Because the majority of current was outward (hence due to an inward flow of chloride ion), these currents would be expected to be reduced in a sizable and predictable amount by reduction of extracellular chloride. In Fig. 2A, currents were recorded using 134, 30, or 8 mM extracellular chloride while keeping intracellular chloride constant at 30 mM. Current records represent the mean of 14 cells (134 mM), 5 cells (30 mM), or 8 cells (8 mM). As expected, reduction of external chloride reduced the outwardly rectifying current. Data are summarized in histogram form in Fig. 2C for current magnitudes resulting from +120-mV membrane depolarization. However, inward currents responded enigmatically to reduction of extracellular chloride. Current magnitudes, expected to be somewhat enhanced, were slightly reduced in magnitude and the reversal potential, expected to be shifted in the depolarizing direction, was shifted in the hyperpolarizing direction. Additionally, these inward currents subsequently proved to be insensitive to most manipulations of the chloride conductance subsequently described. Thus it is likely that these small inward currents could result from a contaminating conductance, such as a cation leak current. Along this line, Doolin and Gilbertson (1996), using rat posterior taste receptor cells, recorded a leak inward current at these potentials that was partially sodium selective. However, it seems unlikely that the leak current recorded in their study is contributing to the inward current in the present study. Doolin and Gilbertson (1996) recorded an inward current of about −120 pA at a holding potential of −70 mV using usual compositions of ECF and ICF. Substituting a sodium-free ECF, this steady inward current was reduced to about −40 pA (or −80 pA of sodium-dependent leak current). The present study differed considerably in both the holding potential (0 mV) and compositions of ECF and ICF. Using a ramp potential, the total inward current, at −70 mV, was only about −10 pA (rather than −120 pA) and was essentially the same in sodium–free ECF (Fig. 3). This suggests that the combination of ionic composition and holding potential used in our experiments substantially eliminated any sodium leak current even before sodium substitution. Additionally, a sodium leak current would be expected to shift the reversal potential in the depolarizing direction (toward the sodium equilibrium potential of about +50 mV), whereas the actual reversal potential shifted in the hyperpolarizing direction. Taken together, the magnitude of the inward current, its insensitivity to the reduction of extracellular sodium, and the direction of the reversal potential shift make sodium an unlikely candidate for any putative leak conductance. Although several potassium-blocking agents were present during the recording, a small inwardly rectifying potassium conductance might possess more appropriate biophysical characteristics as a leak current candidate than sodium. As well, it is not unusual for chloride conductances to deviate from strict Nernstian predictions. Unlike cation channels, anion channels have appreciable cation conductance (e.g., Hille 1992) and in low chloride solutions cation permeability becomes larger and significantly affects the reversal potential (Franciolini and Nonner 1987; Gelband et al. 1996). Taken together, these observations suggest that these inward currents are not an ideally chloride-selective conductance but do not eliminate the possibility that channels with differing biophysical properties and/or leak current may carry the inward currents.

Modulation of intracellular chloride concentration was also performed. Figure 2B presents mean data using two intracellular chloride concentrations of either 30 mM ($n = 12$ cells) or 146 mM ($n = 20$ cells) while keeping extracellular chloride constant at 134 mM. In these experiments the expectations of enhanced inward currents, shift of the reversal potential to close to zero, and diminished outward currents were all observed. However, above membrane potentials of about +50 mV, elevated intracellular chloride concentrations actually enhanced the recorded current. Although the basis of this enhanced current is presently unknown, a recent report that high intracellular chloride concentrations can act to depress G protein modulated ionic conductances may be germane (Lenz et al. 1997). We have studied the G protein cAMP mediated inhibition of potassium currents in these cells (Herness et al. 1997). Depression of baseline inhibition of these potassium currents could result in measurable enhancement of the outward current. Because potassium currents in these cells are on the order of magnitude of −8,000 pA at +120 mV, even a small contribution of potassium current to the outward current could account for the enhancement to 200 pA.

In another set of experiments, chloride currents were recorded in the absence of extracellular sodium to test for potential contributions of the electrogenic Na/K/Cl pump, known to occur in some tissues, or alternatively for contributions of a leak sodium current to the recorded currents. In these experiments, extracellular sodium was replaced with choline while maintaining extracellular chloride at 134 mM. Data are presented in Fig. 3. There was little, if any, difference between current records produced with ramp potentials in these two recording solutions ($n = 4$ cells; Fig. 3A). A small but consistent reduction in the current (~4%) was measured at potential more positive than +50 mV. At +120 mV the current was 142 ± 32 pA in normal sodium ECF and
136 ± 36 pA in choline-replaced ECF (Fig. 3B). Currents were identical at potentials more negative to +50 mV. This observation suggests that sodium contributes little if at all to the total outward current recorded.

Calcium dependence of chloride currents

To test whether chloride currents recorded from taste receptor cells contained a calcium-dependent component, current was evoked before and during presentation of the calcium-channel blockers cadmium (a blocker of a wide variety of calcium channels) or nifedipine (a blocker more specific for L-type calcium channels). Data are presented in Fig. 4. Both calcium channel blockers were effective in reducing the magnitude of chloride currents, suggesting that these currents contain a calcium-dependent component. Cadmium, tested at 1 mM, effectively reduced the outwardly rectifying portion of the current. Inward chloride current was unaffected by the presence of cadmium. This would be expected because calcium currents would not be active at these potentials.

FIG. 2. Chloride dependence of chloride currents. A: whole cell chloride currents recorded with varying external chloride concentrations. Currents were recorded with either 134 mM (●; n = 14 cells), 30 mM (○; n = 5), or 8 mM (■; n = 8) chloride in the bathing solution with 30 mM chloride in the pipette for all cases. Mean data are presented. Chloride currents were reduced with diminished external chloride. Ramp protocol was identical to that presented in Fig. 1. B: whole cell chloride currents recorded with varying internal chloride concentrations. Currents were recorded with either 30 mM internal chloride (●; n = 12 cells) or 146 mM internal chloride (○; n = 20) using 134 mM external chloride for both cases. C: data from A and B presented for currents at +120 mV in histogram form. Currents were depressed with lowered extracellular chloride concentrations or biphasically depressed or enhanced with membrane potential when internal chloride was elevated.

FIG. 3. Sodium independence of chloride currents. A: chloride currents were recorded in standard recording solution or one in which extracellular sodium had been replaced with choline. No obvious differences were noted. B: histogram summary of the chloride current magnitude at membrane potential of +120 mV in response to removal of sodium from the extracellular medium. Data (n = 4 cells) are presented as means ± SE. There was no significant effect of sodium removal on inward current, outward current, or the reversal potential.
potentials. Similarly, the reversal potential was unchanged in the presence of cadmium. Outward currents, however, were reduced up to 48% ($P < 0.019$). Data with nifedipine were similar to those obtained with cadmium. Only the outwardly rectifying portion of the current was affected by nifedipine, tested at 100 $\mu$M (Fig. 4B). Data are means of 3 cells. Neither the inward chloride current nor the reversal potential was affected. Outward current was reduced by up to 21% ($P < 0.015$). Data (mean ± SE) are summarized in Fig. 4C in histogram form for a test pulse to +120 mV. Cadmium reduced the current at this potential from 153 ± 8.8 pA to 80 ± 12.5 pA, whereas nifedipine reduced the current from 157 ± 40 pA to 123 ± 29 pA.

Pharmacological analysis of chloride currents

Chloride current was further characterized by examining the effect of several chloride channel blockers. Four agents were tested: the two stilbene derivatives DIDS and SITS, the fenamate blocker niflumic acid, and the chloride channel blocker 9-anthracene carboxylic acid (9-AC).

FIG. 4. Calcium dependence of chloride currents. A: cadmium, a calcium channel blocker, when applied was extracellularly as effective in reducing the magnitude of the chloride currents (mean data of 3 cells). The outwardly rectifying portion of the current was preferentially inhibited while inward currents were unaffected. B: nifedipine, a specific inhibitor of L-type calcium channels, was similarly effective in reducing the magnitude of the outwardly rectifying portion of the chloride currents. Data are means of 3 cells. C: histogram summary of chloride current magnitude in response to membrane depolarization to +120 mV for both calcium-channel blockers. Data are presented as means ± SE. Cadmium reduced the current by ~48% ($P = 0.019$), whereas nifedipine reduced the current by ~22% ($P = 0.015$).

FIG. 5. Sensitivity of chloride currents to the distilbene inhibitors, DIDS and SITS. A: mean whole cell chloride currents from 5 cells is presented before (●) and during (○) presentation of 500 $\mu$M DIDS. DIDS reduced the magnitude of the outward portion of the current without altering inward current. B: mean chloride current from 3 cells is presented before (●) and during (○) presentation of 500 $\mu$M SITS. SITS similarly affected only outward currents but was less effective than DIDS. C: data are summarized in histogram form (means ± SE) for a test potential to +120 mV. Both agents significantly reduced the current; DIDS reduced the current by ~40% ($P = 0.0014$) and SITS by ~19% ($P = 0.046$). Asterisks indicate statistical significance.
When tested at 500 \( \mu M \), DIDS effectively inhibited the outward portion of the chloride current without noticeable effects on the inward portion (Fig. 5A). The effect could be partially washed out, with removal of DIDS from the bathing solution, but recovery was never complete, in agreement with the observations of others (Ackerman et al. 1994; Ullrich and Sontheimer 1996). Peak current, at +120 mV, was inhibited from 116 ± 0.9 pA to 70 ± 0.7 pA, or about a 40% inhibition (Fig. 5C, mean ± SE of 5 cells; \( P = 0.0014 \)). Inhibition was most effective at positive depolarization potentials, whereas it was ineffective at negative membrane potentials. DIDS was similarly effective in inhibiting the outward current in the presence of 0.5 mM cadmium ([Cl]_{in}/[Cl]_{out} = 30/134 mM; \( n = 5 \), data not shown), again with no effect on the inward portion of the current. This suggests that at least some of the current inhibited by DIDS is calcium independent. SITS, another distibene inhibitor, was less effective than DIDS in reducing the magnitude of the evoked current. When tested at 500 \( \mu M \) in K–free ECF, outward, but not inward, currents were inhibited (Fig. 5B, \( n = 3 \) cells). Outward current was inhibited from 155 ± 0.9 pA to 126 ± 0.2 pA, or about a 19% inhibition (Fig. 5C; \( P = 0.046 \)). The reversal potential was unaltered.

**FIG. 6.** Actions of the chloride channel inhibitors niflumic acid and 9-AC. A: niflumic acid (500 \( \mu M \)) was an effective inhibitor of the outwardly rectifying portion of the evoked currents. Mean data from 5 cells are presented. B: increasing the concentration of niflumic acid from 500 to 1,000 \( \mu M \) did not substantially increase the inhibition of the outward chloride currents. However, small inhibitions of the inward currents were produced (○). Data are means of 7 cells. C: data summarized in histogram form (mean ± SE) for a test pulse to +120 mV as the percentage of remaining (i.e., uninhibited) current. Niflumic acid at 500 and 1,000 \( \mu M \) significantly inhibited the current by 43% (\( P = 0.024 \)) and 40% (\( P = 0.00038 \)), respectively. The chloride channel inhibitor 9-AC was ineffective in reducing outward current. Asterisks indicate statistical significance.

**FIG. 7.** Isoproterenol (ISP) enhancement of chloride currents. A: chloride currents, recorded in response to a ramp potential before (○) and during (●) presentation of 100 \( \mu M \) ISP in the bathing solution. The outwardly rectifying portion of the current was enhanced in the presence of ISP. Data are the mean of 8 cells. B: currents were recorded in reduced extracellular chloride to verify that the enhanced current was carried by chloride. Current was recorded in standard bathing solution (●; [Cl]_{out} = 134 mM), reduced extracellular chloride (○; [Cl]_{out} = 8 mM), and reduced extracellular chloride with 100 \( \mu M \) ISP (▲; [Cl]_{out} = 8 mM). ISP failed to enhance the current when extracellular chloride was reduced. C: summary of the data in A in histogram form. ISP significantly increased the chloride current to a +120-mV membrane depolarization by ~28% (\( P = 0.000016 \)). D: summary of data to current responses to +120 mV from B. Reducing extracellular chloride from 134 to 8 mM significantly reduced the current by ~57% (\( P = 0.006 \)) and was subsequently unaffected by presentation of ISP (\( P = 0.008 \)). Data are presented as means ± SE. Asterisks indicate statistical significance.
Niflumic acid was an effective inhibitor of the outwardly rectifying portion of the chloride currents. Data are presented as both 500 and 1,000 μM (Fig. 6, A and B). At a test pulse of +120 mV (Fig. 6C), current was inhibited by 42 ± 9.5% by 500 μM niflumic acid (n = 5 cells; P = 0.024) and by 40 ± 6.1% at 1,000 μM (n = 7 cells; P = 0.00038). Data for 1,000 μM niflumic acid was obtained using a chloride gradient of 134/146 (out/in), which explains why the baseline current is larger in Fig. 6B than in 6A (as was also observed in Fig. 2B).

Finally, the agent 9-AC appeared to be ineffective on these currents. 9-AC was tested at both 100 and 500 μM without effect. Data are summarized in Fig. 6C (n = 2 cells). Mean response during the presence of 9-AC was 99.8 ± 0.02% (n = 2 cells). Because this agent required solubilization in 0.01% DMSO before preparation in solution, DMSO alone was tested (0.01%) and also determined to be without effect on these currents. We have similarly determined that DMSO by itself at this concentration does not affect outward potassium currents in these cells (Herness et al. 1997).

Isoproterenol enhancement of outward chloride currents

In several cell types, such as cardiac myocytes (Harvey 1996) and amphibian epithelium (Larsen et al. 1995; Willumsen et al. 1992), chloride currents are known to be positively modulated by noradrenergic stimulation, generally via β-receptor activation. We tested this possibility in taste receptor cells using the β-receptor agonist isoproterenol (ISP) and subsequently with the β-receptor antagonist propranolol. When tested at 100 μM, ISP effectively enhanced the outward portion of the chloride current in a total of 15 tested cells (Figs. 7A, 8A, and 9A). Currents were measured as previously de-
scribed by a ramp protocol before and during application of 100 μM ISP (Fig. 7A, n = 8 cells; P = 0.000016). Peak current, measured at +120 mV, was enhanced to 128 ± 10.7% of control values (Fig. 7C, n = 8 cells). Enhancement was only observed for the outwardly rectifying portion of the current. Inward chloride current remained unaffected by application of ISP.

To verify that the enhanced conductance was carried by chloride ions, an ion substitution experiment was performed. Extracellular chloride was lowered from 134 to 8 mM. The reduction of extracellular chloride inhibited outward and, to a lesser degree, inward chloride currents, essentially replicating the data presented in Fig. 2A (42% inhibition; P = 0.006). Subsequently, ISP was presented in low extracellular chloride and now failed to significantly modify the magnitude of the evoked current (Fig. 7B, n = 4 cells; P = 0.0085). Current, at 120 mV was 90 ± 4.5 pA in low chloride and 76 ± 4.7 pA in low chloride plus ISP (Fig. 7D).

Experiments were performed to test for the calcium dependence of the ISP enhancement of chloride currents. In two separate sets of experiments, cadmium was added to ISP either after ISP administration (Fig. 8A) or before its administration (Fig. 8B). With the use of the former protocol, ISP was first demonstrated to be effective in enhancing the outward currents (Fig. 8A; n = 4 cells). However, when cadmium (1 mM) was added to the bathing solution in the sustained presence of ISP, the currents were diminished to below control levels. These data are consistent with those presented in Fig. 4A, where cadmium similarly reduced chloride current magnitude. Similar results were obtained when cadmium administration was applied before ISP administration. Here cadmium administration eliminated the calcium-activated component of the chloride current (Fig. 8B; 32% inhibition; P = 0.012). When ISP was subsequently applied, no enhancement was observed (38% inhibition of control currents; P = 0.016). The reductions of the control current were to 100 ± 21 pA for cadmium and 95 ± 20 pA for cadmium in the presence of ISP. These data suggest that the ISP enhancement is calcium dependent.

In a final set of experiments, the specificity of receptor mechanism of the ISP enhancement was tested. ISP enhancement, if operating through β-receptors, should be prevented by prior exposure to a β-receptor antagonist. Propranolol, a commonly used adrenergic antagonist specific for β-receptors, was employed to test this hypothesis. Data are presented in Fig. 9. Cells were first exposed to 100 μM ISP to measure enhancement of the current then subsequently exposed to 10 μM propranolol followed by an ISP/propranolol mixture. In two cells, ISP application was tested again after propranolol exposure, if recording conditions permitted. In the tested cells, ISP enhanced the current as previously observed. (1.40 ± 0.87%; n = 4; P = 0.016; data normalized to preexposure control current). However, in the presence of propranolol, ISP was ineffective in altering the magnitude of the evoked current (0.98 ± 0.022) suggesting the ISP effect to occur through β-receptors. Control current and ISP/propranolol current were essentially indistinguishable. These normalized data are summarized in Fig. 9B. In two cells, the recording session lasted long enough to allow a second application of ISP. In both of these cells, the second ISP application increased the evoked current, although not to the original degree (1.12 ± 0.02). Both
rundown and residual binding of propranolol may contribute to the diminished effect of the second ISP exposure.

**Discussion**

Gustatory transduction mechanisms require the complex coordination of intracellular signaling cascades that ultimately influence cellular excitability by modulating a variety of ion channels. Fundamental to the detailed understanding of these mechanisms is the careful examination of individual ionic conductances expressed in taste cells. In our studies of posterior taste receptor cells, we have examined a variety of cationic conductances, such as voltage-dependent sodium currents (Herness and Sun 1995), outward potassium currents, including delayed-rectifier, transient, and calcium-activated currents (Chen et al. 1996), and inwardly rectifying potassium currents (Sun and Herness 1996b). With the exception of inwardly rectifying potassium currents, these currents are heterogeneous in their distribution across taste cells, just as the chemical sensitivity of individual taste cells varies on a cell-by-cell basis. Anionic currents, however, have not been described. Because chloride currents subserve diverse functions in other cell types, including the modulation of cellular excitability, their examination is essential toward understanding the physiology of taste receptor cells.

**Types of chloride currents in posterior taste receptor cells**

Taste receptor cells, which are differentiated epithelial cells, appear to possess a heterogeneous array of chloride conductances that are typical to many epithelia. Prominent features of the whole cell chloride conductance in taste cells include pronounced outward rectification, calcium dependence of a portion of the current, measurable conductance near the resting potential, and putative modulation by adrenergic agents.

Epithelia typically possess multiple chloride conductances, several of which are outwardly rectifying and/or calcium dependent (e.g., Frizzell and Morris 1994; Valverde et al. 1995). Three major chloride conductances in epithelia include a cAMP-dependent current, a calcium-dependent current, and a volume-sensitive current important in regulatory volume decrease (RVD). Each display some unique biophysical properties. For example, the cAMP-dependent current is mostly linear in its I-V properties, is not calcium dependent, and is DIDS insensitive. This current in epithelia is often carried by the CFTR channel or an isoform of it (Anderson et al. 1992). Conversely, the calcium-dependent and volume-sensitive currents are both outwardly rectifying; however, the former often displays a time-dependent increase in current magnitude with maintained depolarization, whereas the latter displays a time-dependent decrease in current magnitude.

In taste receptor cells, most of the chloride conductance is outwardly rectifying, that is likely composed of linear and rectifying components. A portion of this current is calcium dependent, as indicated in Fig. 4. In the presence of either calcium inhibitors or chloride channel blockers, such as DIDS, the current loses much of its rectification, leaving a remainder current more linear in nature. The linear nature of the DIDS-insensitive current, and the adrenergic enhancement of the whole cell current (next section) are suggestive, but not conclusive, evidence for a cAMP-dependent current.

**Adrenergic modulation of chloride currents**

Data obtained with the β-adrenergic agonist ISP and antagonist propranolol suggest that an adrenergic enhancement of chloride current may be occurring in taste receptor cells. This observation, which is a novel observation to taste receptor cells, is similar to the well-documented adrenergic modulation in other systems such as cardiac myocytes and amphibian skin.

In cardiac myocytes, two chloride currents are activated or enhanced by β-stimulation (Harvey 1996): a cAMP-regulated current similar to CFTR, which is directly activated by cAMP, requires protein kinase A (PKA)–dependent phosphorylation, and acts to shorten the action potential, and a calcium-dependent chloride current, which is likely indirectly activated by an enhancement of an L-type calcium current. Adrenergic enhancement of chloride current also occurs in amphibian epithelium. Two cell types (the principal cells and the mitochondria–rich cells) work in concert to transport salt. The mitochondria-rich cells transport chloride specifically, and this chloride transport is enhanced by ISP in a manner mediated by cAMP (Larsen et al. 1995; Willumsen et al. 1992).

The underlying mechanism of adrenergic enhancement of chloride current in taste cells remains to be elucidated. It is presumed, but presently untested, that such enhancement would be cAMP dependent, because ISP is a β-receptor agonist and β-receptors operate through G protein stimulation of adenylate cyclase. Alternatively, because the ISP-enhanced current was determined to be calcium dependent, ISP could enhance calcium currents and secondarily enhance the calcium-dependent chloride currents. Such enhancement of calcium currents by ISP was recently reported in the amygda (Huang et al. 1998).

ISP has other actions on different ionic conductances in taste cells. In taste cells we have observed that ISP also inhibits outward potassium currents. This mechanism appears to be mediated by cAMP and PKA phosphorylation (Herness et al. 1997; Sun and Herness 1996a). Additionally, recent evidence suggests that cAMP inhibits voltage-dependent sodium currents (Herness 1997). Thus ISP-mediated elevations of cAMP might be expected to additionally modulate sodium channels. These additional actions of ISP on other currents are also observed in cardiac tissue. In response to sympathetic stimulation of the heart, β-adrenoceptor activation also stimulates an L-type Ca current, the pacemaker current, delayed rectifier current, and inhibits sodium current (Harvey 1996).

The source of norepinephrine within the taste bud is presently unknown. There is some evidence that adrenergic nerve fibers surround taste buds (i.e., perigemmal nerve fibers) (Papparelli et al. 1986). The density of these fibers seems to vary from species to species, and they may be branches from perivascular axons. Additionally, stimulating the sympathetic nerve supply to the tongue or systemic injections of epinephrine was shown to enhance taste responses recorded in the chorda tympani nerve in rats (Kimura 1961). In the frog, norepinephrine (but not dopamine) injected in the lingual artery was reported to enhance glossopharyngeal nerve responses to gustatory stimuli (Morimoto and Sato 1982; Nagahama and Kurihara 1985).

In general, actions of neurotransmitters on taste receptor cells are not yet well studied. Evidence thus far suggests that their actions may be complex. Along these lines, we have
observed that another neurotransmitter, serotonin, actually decreases the calcium-activated potassium current and that this inhibition is probably due to 5HT1A receptors (Herness and Chen 1997). Similarly, it is not yet known if the action of serotonin is directly on calcium-activated potassium current or if it acts indirectly through calcium currents.

**Physiological role for chloride currents**

Chloride currents likely contribute to multiple physiological roles in taste receptor cells that include housekeeping functions such as volume regulation, maintenance of the resting potential, and contributions to cellular excitability. Precise roles in taste receptor cells remain to be elucidated. One of the most alluring is the possibility that chloride currents may play active roles in taste transduction processes. As a comparison, chloride currents are known to play an integral role in transduction of olfactory stimuli (e.g., Kurahashi and Yau 1994).

Because taste receptor cells and epithelial cells appear to share similar distributions of chloride channels, their functions may also be similar. Chloride currents play integral roles in salt transport across epithelia. Similar transport across the lingual epithelium is known (DeSimone et al. 1981, 1984; Miers et al. 1996), and the participation of chloride conductance has been suggested (Wladkowski et al. 1998). Additionally, the apical ends of taste cells are routinely subjected to both hypothonic and hypertonic extremes of osmotic pressure. RVD is present in both excitable and inexecitable cells (reviewed in Nilius et al. 1996; Okada 1997). Interestingly, the volume activated can be blocked by quinine (Voets et al. 1996), a commonly employed tastant prototypic for bitter stimuli. However, an important difference between taste receptor cells and other epithelia cells is that taste receptor cells are electrically excitable. Here, taste receptor cells may be more similar to neurons and cardiac cells where calcium-activated chloride channels participate in modulation of action potential (Harvey 1996; Scott et al. 1995). We have observed that the shape of the gustatory action potential is modulated by DIDS (personal observation), suggesting that chloride currents may contribute to the repertoire of voltage-dependent conductances underlying the action potential. DIDS treatment broadened the duration of the action potential and reduced the magnitude of the afterhyperpolarization. Collectively, these observations suggest that chloride channels may be multifunctional in taste receptor cells.

The ultimate effect that adrenergic modulation of chloride currents would have on the electrical state of the cell is complex and would be influenced by the dynamic state of the membrane potential. For example, adrenergic modulation of a taste cell at rest would be expected to bring the membrane potential closer to the chloride equilibrium potential and likely act to stabilize the membrane potential. Under these conditions, the increase of cAMP due to activation of β-receptors would not alter potassium conductances because, at rest, most of these conductances are closed. We have observed that cAMP does not depolarize the resting potential of posterior taste cells (Herness et al. 1997), consistent with the notions that there is no closure of a resting potassium conductance and that putative enhancement of the chloride current might be stabilizing the membrane potential. An additional complication lies in defining the resting potential of taste cell and establishing the true chloride reversal potential, which is altered in the experimental situation by the choice of extracellular and intracellular chloride ion concentrations. The conventional values of 134 and 30 mM for external and internal chloride establishes a reversal potential of about −35 mV, which would likely be a depolarizing influence for many taste cells if the resting chloride conductance were enhanced. However, we have observed mean zero-current potentials for posterior taste cells of −37 mV with a range of 0 to −85 mV (Herness and Sun 1995, Table 1). On the other hand, if the cell were in an active state, i.e., firing an action potential, norepinephrine release would be expected to decrease an outward potassium current and increase an outward chloride current in those taste receptor cells expressing β-receptors. Such actions would result in a prolonged duration of the action potential, due to inhibited outward potassium currents, and possibly an exaggerated afterhyperpolarization, due to the contribution of chloride currents, causing an adaptation of the firing rate. In short, the interactions of currents regulating the electrical excitability of the cell are complex, and such dynamic factors as the active state of the cell and the temporal phase of the membrane potential must be considered to understand influences of modulatory actions. Given the heterogeneity of membrane properties and the diversity of chemosensitivity of taste receptor cells, generalizations regarding the properties of membrane conductances are of limited value. Correlations of chemosensitivity, ion channel repertoire, and expression of transmitters and their receptors within single cells will be necessary to fully understand the roles of neurotransmitters within the taste bud.

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