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A Basolateral Chloride Conductance in Rat Lingual Epithelium

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Abstract. We used Ussing chamber measurements and whole-cell recordings to characterize a chloride conductance in rat lingual epithelium. Niflumic acid (NFA) and flufenamic acid (FFA), nonsteroidal anti-inflammatory aromatic compounds known to inhibit Cl^- conductances in other tissues, reduced transepithelial short-circuit current (I_{sc}) in the intact dorsal anterior rat tongue epithelium when added from the serosal side, and reduced whole-cell currents in rat fungiform taste cells. In both Ussing chamber and patch-clamp experiments, the effect of NFA was mimicked by replacement of bath Cl^- with methanesulfonate or gluconate. In low Cl^- bath solution, the effect of NFA on whole-cell current was reduced. Replacement of bath Ca^{2+} with Ba^{2+} reduced the whole-cell Cl^- current. We conclude that a Ca^{2+}-activated Cl^- conductance is likely present in the basolateral membrane of the rat lingual epithelium, and is present in the taste receptor cells from fungiform papillae. Further experiments will be required to identify the role of this conductance in taste transduction.

Key words: Taste — Chloride conductance — Lingual epithelium — Niflumic acid — Flufenamic acid — DIDS

Introduction

Chloride channels are expressed in many cell types, where they serve a variety of physiological functions (for review, *see* Hille, 1992). In skeletal muscle, Cl^- channels comprise the dominant leak conductance and contribute to stabilizing the resting potential. Chloride channels also play a clearly defined role in epithelia, where they are involved in volume regulation and in controlling the flux of salt and fluid between body compartments. Chloride channels are also present in sensory cells, where a Ca^{2+}-dependent Cl^- conductance participates directly in olfactory transduction. Odorants bind to receptors that activate cAMP; the cAMP then gates a cation channel, causing Ca^{2+} influx. The Ca^{2+} in turn gates a Cl^- channel, which elicits Cl^- efflux and membrane depolarization (Kleene & Gesteland, 1991; Kurahashi & Yau, 1993). Recently, cAMP has been found to gate a Cl^- channel directly in *Necuturus* olfactory receptor neurons (Delay, Dubin & Dionne, 1997).

The role of chloride conductances in the lingual epithelium has not been extensively studied. The lingual epithelium contains both taste buds, onion-shaped aggregations of taste receptor cells, and nongustatory epithelial cells. Although the taste receptor cells are the transducing elements of gustatory sensation, current flow through the nongustatory epithelium may also contribute to taste transduction (Elliot & Simon, 1990; Ye, Heck & DeSimone, 1991; Simon et al., 1993; Harper, 1994). McBride and Roper (1991) and Taylor and Roper (1994) identified a Ca^{2+}-dependent Cl^- conductance in the basolateral membrane of *Necturus* taste cells. This conductance is responsible for producing the large after potentials that are observed in these cells following action potentials elicited by taste stimuli. Because this conductance would tend to limit repetitive firing of action potentials, it was hypothesized to play a role in taste cell adaptation.

Until recently, chloride channels have not been reported in mammalian taste cells (Wladkowski et al., 1995; Sun & Herness, 1995). Based on the chorda tympani response to NaCl stimuli, Elliott and Simon (1990) failed to show any effect of chloride transport inhibitors (0.1 mM SITS, DIDS, furosemide, bumetanide, 9-anthracene carboxylic acid, or an antibody that blocks

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Cl^- conductance pathways in many epithelia) applied to the mucosal side of the rat tongue in vivo. Similar results were obtained in hamster (Rehnberg et al., 1993). In constructing a mathematical model of the tongue epithelium, Mierson and Fidelman (1994) hypothesized that Cl^- conductances and transporters would be present in the basolateral membrane. In the canine dorsal anterior tongue epithelium in vitro (Ussing chamber experiments), removal of Cl^- from the serosal solution affected transepithelial I_{sc}, relative to the value in symmetrical 0.15 M NaCl (Mierson et al., 1985).

In this study, we combined voltage clamp experiments in an Ussing chamber with whole-cell recordings from single taste cells to characterize Cl^- conductance in the rat lingual epithelium. The polarity of the tissue is preserved in Ussing chamber experiments, so that inhibitors can be applied selectively to either the basolateral or apical membrane. In addition, solutions with a wide range of osmolarity can be applied to the mucosal side in order to approximate physiological conditions in vivo. However, since the lingual epithelium is a heterogeneous population of cells, this method cannot distinguish what type of cell is being affected by an inhibitor. Thus, whole-cell recording was used to determine the effects of inhibitors on individual taste cells. A potential caveat in this approach is that taste buds make up only a small percentage of the lingual epithelium. Thus, Ussing chamber measurements would be expected to be dominated by current flow through the nongustatory epithelium. Denervation experiments, however, suggest that about 20% of the current flux across the lingual epithelium results from current flow through the taste buds (Simon et al., 1993). Ussing chamber measurements should be able to provide a qualitative assessment of the location of Cl channels on the taste receptor cells.

We used an in vitro preparation of the dorsal rat tongue epithelium in the Ussing chamber to measure the electrical properties of the epithelium. In similar experiments on the canine lingual epithelium, a variety of transport inhibitors had no effect from the serosal side of the tissue (Mierson et al., 1985). However, if the tissue was first incubated with collagenase on the serosal side to digest the connective tissue (Mierson et al., 1988*a*), several of the same inhibitors did have an effect (Mierson & DeSimone, *unpublished results*). This implies that without the enzyme dissection, inhibitors had inadequate access to the tissue. Hence in this study, we used an enzyme dissection that removes all of the connective tissue from the epithelium (Mierson, Olson & Tietz, 1996). We used five Cl^- channel inhibitors: NFA (niflumic acid) and FFA (flufenamic acid), which block Ca^{2+}-activated chloride channels or nonselective cation channels (White & Aylwin, 1990; Poronnik, Ward & Cook, 1992); SITS (4-acetamino-4′-isothiocyanostilbene-2,2′-disulfonic acid) and DIDS (4,4′-diisothiocyanostilbene-2,2′-disulfonic acid), which block chloride channels or anion exchangers, or activate nonselective cation channels (Restrepo et al.,1991; Gögelein & Pfannmüller, 1989); and NPPB (5-nitro-2-(2-phenylpropylamino)-benzoic acid), which blocks chloride channels (Wangemann et al., 1986).

Our results suggest the presence of an FFA- and NFA-sensitive basolateral Cl^- conductance in rat lingual epithelium. Patch-clamp studies suggest the conductance is present in taste receptor cells and is Ca^{2+}-activated. Some of these results have been published previously in abstract form (Wladkowski et al., 1995).

Materials and Methods

The methods utilized in this study result from our extensive previous experience with these preparations. Tyrode's solution is traditionally used to maintain stable long-term whole-cell recordings in isolated taste cells. For transepithelial measurements in Ussing chamber experiments on the other hand, Krebs-Henseleit (KH) buffer is traditionally used. We found in conducting this study that when the serosal buffer was changed back and forth between KH and Tyrode's for the same tissue, higher values of current and voltage were consistently obtained with KH. In addition, patch-clamp studies of taste cells have always required room temperature to maintain stable recordings, while transepithelial current and voltage values in the Ussing chamber are lower at room temperature than at 34°C (*vide infra*). Thus, there were some systematic differences in tissue preparation and maintenance for these two preparations. However, as we will discuss later, we do not believe these differences accounted for the differences observed in this study.

Ussing Chamber Experiments

Tissue Preparation

Adult Wistar rats (Hilltop Laboratory) were sacrificed with a lethal injection of sodium pentobarbital *i.p.* The tongue was excised and the epithelium prepared free of underlying muscle and connective tissue by a modification of the methods of Béhé et al. (1989, 1990) as described previously (Mierson et al., 1996). The tongue was injected just under the epithelium with an enzyme solution containing either 1.5 mg/ml pronase E or a mixture of 1.5 mg/ml pronase E and 1.0 mg/ml elastase (Spielman et al., 1989). For experiments with DIDS and SITS, which are soluble in aqueous solutions, the tissue was prepared with pronase alone. Mierson et al. (1996) found that the response to a hydrophobic inhibitor can be reduced and slower if the tissue is prepared with pronase alone as compared to pronase plus elastase. Elastase may affect the glycocalyx (A.I. Spielman, *personal communication*), and thus remove a layer that functions as a diffusion barrier for some substances. For this reason, in experiments using FFA, NFA, or NPPB the tissues were prepared with pronase plus elastase. In a few experiments, the epithelium was prepared using dispase, trypsin inhibitor, and collagenase, the enzymes used to prepare the taste buds for patch-clamp experiments (*vide infra*). Between 0.5 and 1.0 ml of the enzyme solution was injected. After incubating the tongue in Tyrode's buffer bubbled with 100% oxygen for 25 min at room temperature, the epithelium could be easily peeled away from the muscle and connective tissue. The tissue was sandwiched between two silastic gaskets, attached to the gasket on the mucosal side with cyanoacrylate adhesive, and mounted

in a modified Ussing chamber. The cross-sectional area of the tissue was 0.56 cm^2; the volume of the chamber was approximately 5 ml on each side.

Electrical Recordings

The recording apparatus is similar to that described in previous publications (Heck, Mierson & DeSimone, 1984; Mierson et al., 1988*b*; Settles & Mierson, 1993; Mierson et al., 1996). Voltage was measured between calomel electrodes connected in series to 0.9% saline/agar bridges using a Physiologic Instruments automatic voltage clamp. Current was passed through electrodes consisting of Ag/AgCl pellets connected in series with 0.9% saline/agar bridges. To measure short-circuit current (I_{sc}), the tissue was clamped to zero transepithelial voltage. I_{sc} was monitored on a strip chart recorder. Transepithelial resistance (R) was determined by pulsing current (±5 mV) for 1 sec. The potential difference could be calculated using I_{sc} and R, since the I-V curves are linear at the salt concentrations used in this study (DeSimone et al., 1984; Fidelman & Mierson, 1989). The convention used is that positive I_{sc} is in the direction of either cation absorption (mucosa to serosa) or anion secretion (serosa to mucosa).

Experimental Protocols

We examined the effects of the inhibitors both in symmetrical buffer and on the hyperosmotic response. The Cl^- concentrations in the intracellular and interstitial compartments are unknown. These concentrations influence the driving force for transport through a basolateral Cl^- conductance; the driving force may change for different mucosal solutions (Mierson & Fidelman, 1994). In vivo experiments often use a stimulus salt concentration of 0.5 M; hence that concentration was chosen for our Ussing chamber experiments.

After mounting the tissue with KH on both sides and bubbling with 95% O_2/5% CO_2, the tissue was warmed until it reached a steady temperature of 34°C (30–60 min). The tissue was then clamped to zero voltage. The following typical experimental protocol to measure effects of inhibitors was similar to one used previously (Mierson et al., 1985). (i) Measure the electrical parameters in symmetrical buffer. (ii) Record a hyperosmotic response by measuring I_{sc} when the mucosal solution is changed from 0.01 M NaCl to 0.5 M NaCl. (iii) Return to symmetrical KH until I_{sc} reaches steady state. (iv) Repeat (ii) and (iii) to make sure that the hyperosmotic response is reproducible. (v) Add inhibitor to the mucosal or serosal solution either for a specified time period or until I_{sc} reaches steady state. (vi) Repeat the hyperosmotic response to see if there is a change in the magnitude of I_{sc}.

When an inhibitor was tested in symmetrical KH, one hyperosmotic response was completed before adding inhibitor to make sure the tissue was viable. If an inhibitor was tested with 0.5 M NaCl on the mucosal side, then two hyperosmotic responses were performed before adding inhibitor to ensure reproducibility. To obtain a concentration response function with respect to an inhibitor, the lowest concentration of inhibitor was added to the serosal solution. When I_{sc} reached steady state, the next higher concentration of inhibitor was added. This was repeated for all inhibitor concentrations.

To obtain concentration response functions with respect to NaCl, solutions of increasing NaCl concentration (in M): 0.01, 0.025, 0.05, 0.1, 0.2, 0.3, and 0.5 were placed in turn on the mucosal side. After returning to symmetrical KH and reaching steady-state, inhibitor was added to the serosal solution, and the NaCl concentration series repeated on the mucosal side.

To compare the effect of low-Cl^- serosal solution with that of an inhibitor, the inhibitor (usually 100 μM) was added to serosal KH ($[Cl^-]$ = 125 mM) for 5 min. After rinsing with serosal KH and allowing I_{sc} to recover and reach steady-state, the serosal KH was replaced with low Cl^- KH ($[Cl^-]$ = 7.5 mM) for 10 min. In each case the percent change of I_{sc} was determined relative to the value of I_{sc} just prior to that manipulation.

Data are expressed as means ± SE. Statistics are calculated using Student's t-test and two-tailed P values.

Solutions and Reagents

Pronase E was obtained from Sigma Chemical and elastase from Worthington Biochemicals. The inhibitors NFA, FFA, DIDS, and SITS were all obtained from Sigma Chemical. NPPB was the kind gift of Dr. R. Greger. DIDS and SITS were readily soluble in aqueous buffer. Niflumic acid and flufenamic acid were first dissolved in a small amount of ethanol, and then diluted to the appropriate concentration with KH. The final concentration of ethanol in the solutions with 100 μM inhibitor was 0.1% ethanol for both NFA and FFA. NPPB was dissolved in a small amount of dimethyl sulfoxide (DMSO) and then diluted with KH. The final concentration of DMSO in the 10 μM NPPB solution was 0.01%.

Tyrode's buffer consisted of (in mM): 140 NaCl, 5 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid buffer (HEPES), 10 glucose and 10 sodium pyruvate. The pH of Tyrode's was adjusted to 7.4 with NaOH. KH consisted of (in mM): 118 NaCl, 5.6 KCl, 1.9 $CaCl_2$, 1.2 $MgSO_4$, 1.3 NaH_2PO_4, 25 $NaHCO_3$, and 5.6 glucose. Low Cl^- KH consisted (in mM): of 118 Na gluconate, 5.6 KCl, 1.9 $CaCl_2$, 1.2 $MgSO_4$, 1.3 NaH_2PO_4, 25 $NaHCO_3$, and 5.6 glucose. The pH of KH was 7.4 when bubbled with 95% O_2/5% CO_2. All chemicals were reagent grade.

Patch-Clamp Experiments

Isolation of Taste Buds

Fungiform taste buds were isolated from mature Sprague Dawley male rats (6–9 weeks old) by similar methods to those already described for preparation of the epithelium. The rat was sacrificed with CO_2 and its tongue removed. An enzyme mixture containing 2-mg dispase (Grade II; Boehringer Mannheim, Indianapolis, IN), 1-mg trypsin inhibitor (Type I-s; Sigma Chemical, St. Louis, MO) and 1-mg collagenase B (Boehringer Mannheim) in 1-ml normal Tyrode's was injected into the tongue between the lingual epithelium and the muscle layer. The tongue was incubated in Ca^{2+} and Mg^{2+}-free oxygenated Tyrode's for 30 to 35 min, or until the epithelium could be gently separated from the underlying connective tissue. Individual fungiform taste buds were removed from the epithelium by suction with a fire-polished pipette and plated onto glass slides coated with Cell-Tak (Collaborative Research, Bedford, MA).

Patch-Clamp Recording

Voltage-dependent and steady-state currents were monitored by the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981). Patch recording pipettes were fabricated from microhematocrit capillary tubes (Scientific Products, McGaw Park, IL) with a two-stage vertical puller (model PB-7; Narishige, Tokyo, Japan). Pipette resistance was 4–6 megohms when filled with standard pipette solution and 8–10 megohms when filled with low K^+/Cl^- pipette solution. Membrane currents were filtered at 2 KHz and recorded with an Axopatch patch-clamp amplifier (model 1D; Axon Instruments, Foster City, CA). An Indec laboratory computer system (Sunnyvale, CA) was used to apply voltage steps and to analyze voltage-dependent currents. For

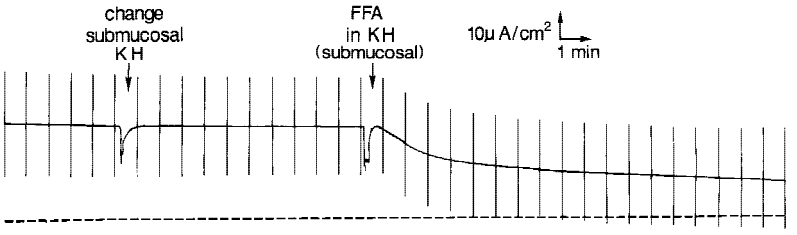

Fig. 1. Typical effects of FFA (100 μM) on I_{sc} in dorsal anterior rat tongue epithelium in vitro. The vertical spikes are current deflections in response to ±5 mV displacements of the clamping potential, and are inversely proportional to transepithelial resistance. The dashed line is the zero point.

steady-state measurements, holding current was recorded at a potential of −80 and 20 mV hyperpolarizing voltage pulses were applied to the pipette to monitor membrane conductance.

Solutions and Stimulus Delivery

Normal Tyrode's was the same as that used for the Ussing chamber experiments. Low Cl^- Tyrode's was identical except that NaCl was replaced with Na gluconate or Na methanesulfonate. The Ca^{2+}-and Mg^{2+}-free Tyrode's used to isolate taste buds contained 2 mM BAPTA (Molecular Probes, Eugene, OR). In some experiments, 100 mM NFA or FFA was added to the bath solution to block the Cl^- conductance. In these experiments, 3 μM TTX, 10 mM 4-AP, 30 μM amiloride and 10 mM tetraethylammonium chloride (TEA) were present in the bath solution to block conductances other than Cl^-. Bath solutions were gravity-fed into the 0.5 ml recording chamber. Flow rates were typically 4–5 ml/min, permitting complete solution exchange in less than 10 sec.

Three different intracellular pipette solutions were used. The standard pipette solution contained (in mM): 134 KCl, 7.45 $CaCl_2$, 1 $MgCl_2$, 10 HEPES (pH 7.2 with KOH), 10 EGTA, 1 ATP, and 0.4 GTP. Low Cl^- pipette solution contained 134 K gluconate in place of KCl. Low K^+/Cl^- pipette solution contained 134 N-methyl-D-glucamine (NMDG), 134 methanesulfonic acid ($MeSO_3$), 10 NaCl, 0.23 $CaCl_2$, 0.1 EGTA, 2 $MgCl_2$, 10 HEPES (pH 7.2 with Tris OH), 1 ATP, and 0.4 GTP.

Results

Ussing Chamber Experiments

Flufenamic Acid and Niflumic Acid

Flufenamic acid in the serosal solution reduced I_{sc} in symmetrical KH (Figs. 1 and 2). The effect was reversible: when FFA was placed on the serosal side of the tissue for 5 min, in the presence of KH on the mucosal side, the I_{sc} response recovered completely within 30–45 min after removing the inhibitor. Niflumic acid similarly inhibited I_{sc} and this effect was also reversible within the same time period. This decrease in I_{sc} in symmetrical KH caused by NFA was accompanied by a small but significant increase in R ($\Delta R = 4.6 \pm 1.4\%$, $P = 0.02$). FFA had no significant effect on R ($5.5 \pm 2.3\%$, $P = 0.1$). To determine the apparent inhibition constant K_i, the concentration of inhibitor that produces half maximal inhibition, the concentration of the inhibitor was varied from 1 to 1000 μM, with KH present on the mucosal side.

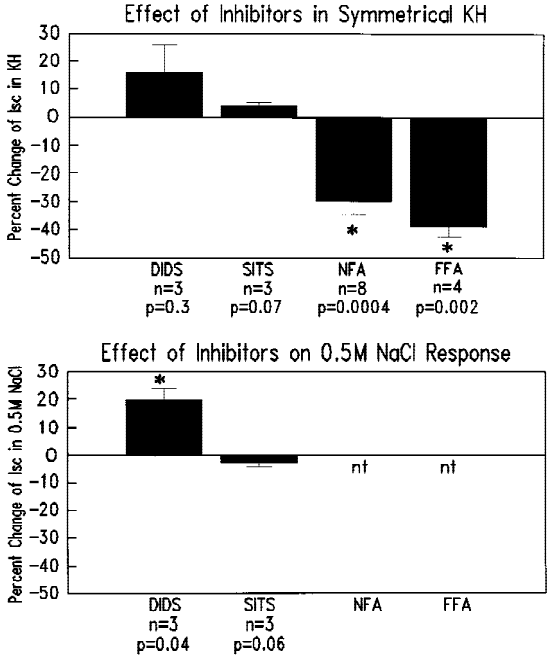

Fig. 2. Effects of inhibitors on I_{sc} in symmetrical Krebs-Henseleit buffer (KH) (top) and with 0.5 M NaCl on the mucosal side (bottom). Values are means ± SEM. Values marked * are statistically significant for a two-tailed P value of $P < 0.05$ using Student's t-test. All inhibitors were 100 μM, except FFA which was 70 μM, and all were added to the serosal side of the tissue; *nt* = not tested using this protocol.

From the concentration-response functions for FFA and NFA, the K_i for FFA was 69 μM, while the K_i for NFA was 329 μM (Figs. 3 and 4).

Both FFA and NFA inhibited I_{sc} at all mucosal NaCl concentrations (Figs. 5*A* and 6*A*). Using this protocol for obtaining a concentration-response function with respect to NaCl concentration, neither NFA nor FFA had any significant effects on R (Figs. 5*B* and 6*B*).

The inhibition of I_{sc} by FFA or NFA was mimicked by replacing serosal NaCl with NaGluconate; gluconate is a large impermeant anion that ordinarily does not permeate Cl^- channels. In sample experiments, I_{sc} in symmetrical KH was decreased by serosal NFA (22%), FFA (39%), or low Cl^- (range of 39–68%). Adding the inhibitor to the low Cl^- bath solution further decreased I_{sc} (*data not shown*).

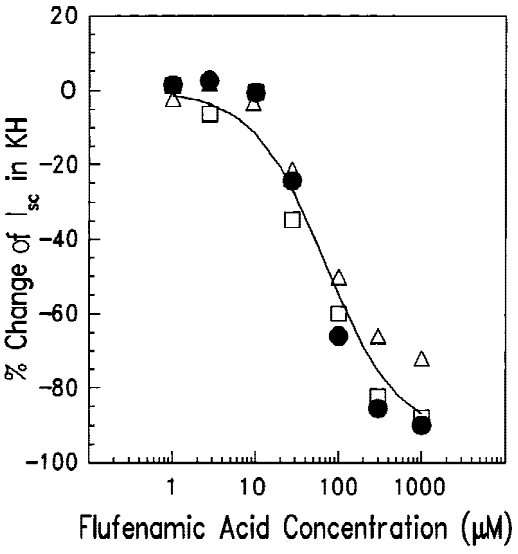

Fig. 3. Flufenamic acid concentration response function: percent change of steady-state I_{sc} in symmetrical KH with respect to serosal FFA concentration. Each symbol refers to a different experiment (n = 3). The curve was fitted to the equation: % change in $I_{sc} = -cx/(K_i + x)$ where c is the maximal percent change and K_i is the FFA concentration corresponding to half maximal percent change under this set of experimental conditions, $K_i = 69 \pm 11$ μM and $c = 93\%$ ($r^2 = 0.96$).

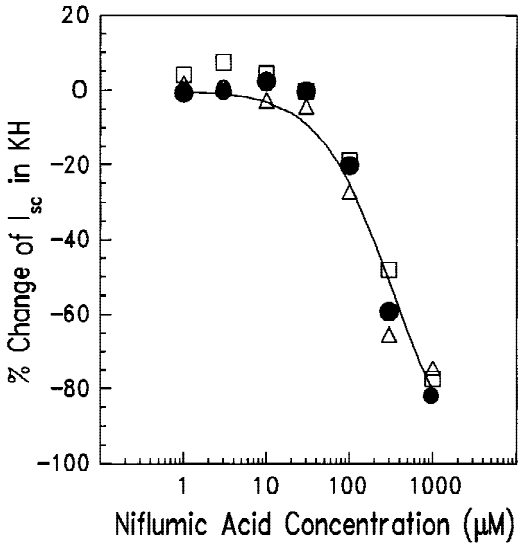

Fig. 4. Niflumic acid concentration response function: percent change of steady-state I_{sc} in symmetrical KH with respect to serosal NFA concentration. Each symbol refers to a different experiment (n = 3). The curve was fitted to the equation: % change in $I_{sc} = -cx/(K_i + x)$ where c is the maximal percent change and K_i is the FFA concentration corresponding to half maximal percent change under this set of experimental conditions, $K_i = 329 \pm 68$ μM and $c = 107\%$ ($r^2 = 0.97$).

Mucosal 100 μM NFA and FFA were also tested. Neither inhibitor had any effect from the mucosal side, either in symmetric KH or in the presence of 0.5 M mucosal NaCl.

DIDS and SITS

Since DIDS and SITS have been shown to block the Cl^- conductance in *Necturus* taste cells (Taylor & Roper, 1994), we tested them on the rat lingual epithelium. DIDS in the serosal solution increased transepithelial I_{sc}: serosal DIDS (100 μM) slightly increased I_{sc} in symmetrical KH, and significantly increased I_{sc} with 0.5 M NaCl in the mucosal solution (Fig. 2). Serosal DIDS had no significant effect on R in symmetrical KH (11.5 ± 6.6%, $P = 0.2$) or in 0.5 M NaCl (0 ± 4%). To determine the apparent activation constant K_a[1] for DIDS, the concentration of DIDS was varied from 1 to 500 μM, in the presence of mucosal 0.3 M NaCl to achieve a large effect for DIDS on I_{sc}; the K_a was 247 μM DIDS (Fig. 7). The increase in I_{sc} was dependent on the mucosal NaCl concentration. The percent increase of I_{sc} was enhanced at higher NaCl concentrations (Fig. 8).

Unlike NFA and FFA, the effect of DIDS on I_{sc} was not mimicked by replacement of serosal bath Cl^- with gluconate. DIDS alone in the serosal solution increased I_{sc} with 0.5 M NaCl present in the mucosal solution. Replacement of serosal bath chloride by gluconate caused a decrease in I_{sc}. Subsequently, adding DIDS to the low Cl^- serosal solution again caused an increase in I_{sc} (*data not shown*). In contrast, FFA caused a decrease in I_{sc}, in the presence of either normal Cl^- or low Cl^-.

Serosal 100 μM SITS had no significant effect on I_{sc} or R, either in symmetrical KH or with 0.5 M NaCl in the mucosal solution. NPPB, 10 μM, had no effect from either mucosal or serosal side. With 0.3 M NaCl in the mucosal solution, serosal 100 μM NPPB decreased I_{sc} by 100 ± 3.8% ($n = 4$). Although the data for 100 μM are statistically significant ($P < 0.05$), these results were not taken as evidence for the presence of a chloride conductance, because at that concentration NPPB can have nonspecific effects (P. Wangemann, *personal communication*).

PATCH-CLAMP EXPERIMENTS

Whole-cell voltage-clamp recordings were obtained from single taste receptor cells in isolated rat fungiform

[1] An increase in I_{sc} can be due to either an increase in cation transport in the M to S direction, a decrease in cation transport in the S to M direction, a decrease in anion transport in the M to S direction, or an increase in anion transport in the S to M direction. Although the overall effect of DIDS is to increase I_{sc} and hence we use the term K_a, it is not known whether the effect of DIDS on a specific transport step in the lingual epithelium is inhibitory or stimulatory.

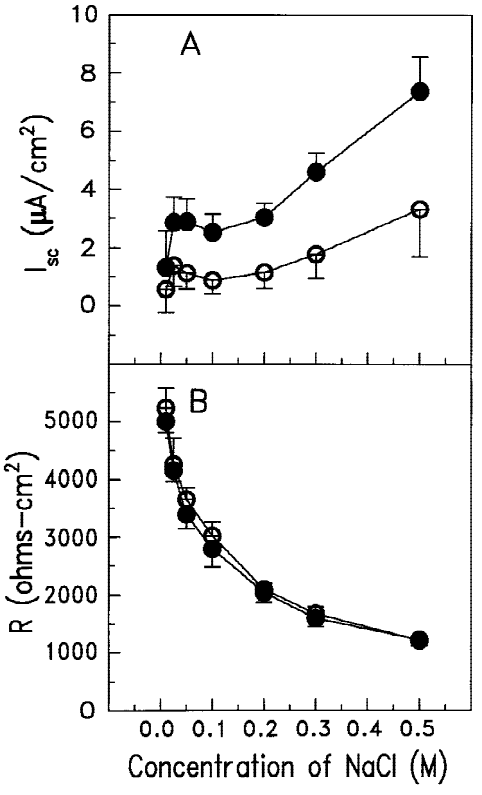

Fig. 5. Steady-state values of I_{sc} (*A*) and *R* (*B*) over a concentration range from 0.01 to 0.5 M NaCl in the mucosal solution, before (●) and after (○) addition of serosal FFA. Values are means ± SEM, $n = 3$. FFA inhibited I_{sc} at all NaCl concentrations.

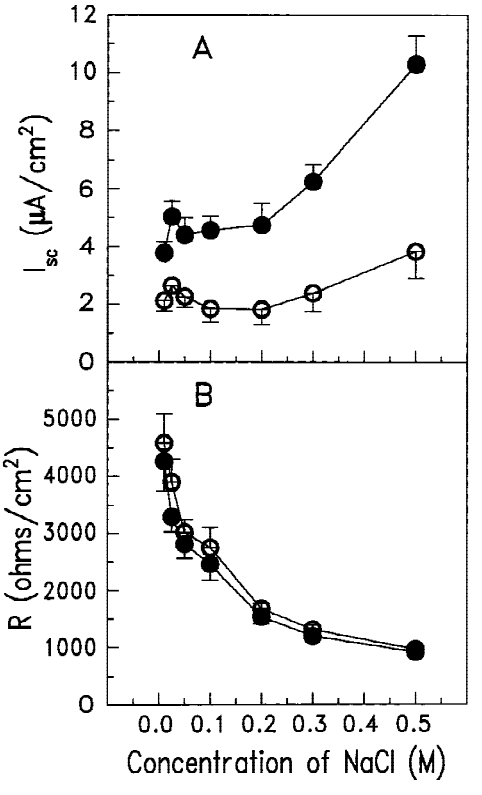

Fig. 6. Steady-state values of I_{sc} (*A*) and *R* (*B*) over a concentration range from 0.01 to 0.5 M NaCl in the mucosal solution, before (●) and after (○) addition of serosal NFA. Values are means ± SEM, $n = 3$. NFA inhibited I_{sc} at all NaCl concentrations.

taste buds. When cells were bathed in normal Tyrode's and the pipette contained standard intracellular solution, a depolarizing voltage pulse to +80 mV from a holding potential of −80 mV elicited a sustained outward current (Fig. 9*A*, top trace). When the bath solution was replaced by low Cl^- Tyrode's, the outward current was reduced by approximately 40%, suggesting that a portion of the outward current was carried by Cl^- (Fig. 9*A*, lower trace). The remaining outward current was sensitive to TEA and 4-AP, indicating that this current was carried by K^+ (*data not shown*). To examine the Cl^- currents in isolation and under more physiological conditions (low intracellular Cl^-), the KCl in the pipette solution was replaced by NMDG and $MeSO_3$. The intracellular Ca^{2+} buffer EGTA was also decreased to allow more Ca^{2+} to accumulate in the cell, thus potentiating any possible Ca^{2+}-dependent component of the Cl^- current. Under these conditions, most cells (18 out of 26) still exhibited outward currents in response to depolarizing voltage steps from a holding potential of −80 mV (Fig. 9*B*). These currents ranged from 50 to 500 pA at +80 mV. The outward currents under these conditions were insensitive to TEA (data not shown), but were reduced or abolished when the bath solution was replaced by low Cl^- Tyrode's (Fig. 10*A*). These data suggest that most rat taste cells contain a Cl^- conductance. To determine if the Cl^- conductance was Ca^{2+}-dependent, Ca^{2+} in the bath was replaced with 5 mM Ba^{2+}, which should not activate Ca^{2+}-dependent conductances. In 16 of the 18 cells tested, replacing Ca^{2+} with Ba^{2+} abolished most or all of the outward current (Fig. 10*B*), suggesting that the outward Cl^- current was a Ca^{2+}-dependent Cl^- conductance.

We examined the effects of NFA on the Cl^- conductance in rat taste cells. Steady-state holding current was recorded at −80 mV and membrane conductance was monitored by applying 20 mV hyperpolarizing voltage pulses to the pipette at 5-sec intervals. The bath solution contained 3 μM TTX, 30 μM amiloride, 10 mM TEA and 10 mM 4-AP to block conductances other than the Ca^{2+}-dependent Cl^- conductance. In 24 of the 35 taste cells tested, NFA (100 μM) decreased the inward holding current with a concomitant decrease in the membrane conductance (Fig. 11, upper trace). For the low Cl^- solution, NaCl was replaced with Na gluconate (Fig. 9) or with Na methanesulfonate (Figs. 10 and 11); the results were identical for either solution.

Flufenamic acid in whole-cell recordings showed

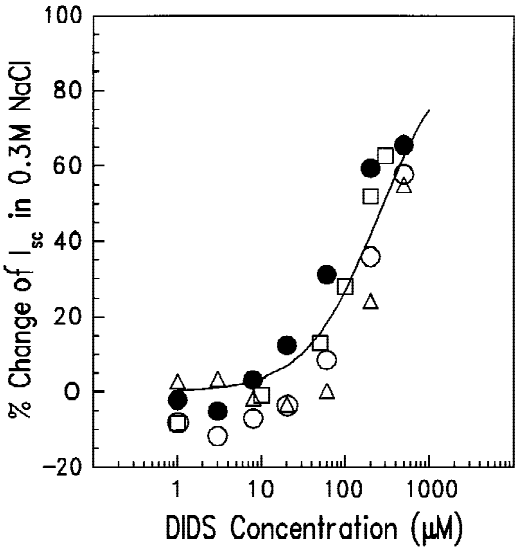

Fig. 7. DIDS concentration response function: percent change of steady-state I_{sc} in symmetrical KH with respect to serosal DIDS concentration. Each symbol refers to a different experiment ($n = 3$). The curve was fitted to the equation: % change in $I_{sc} = cx/(K_a + x)$ where c is the maximal percent change and K_a is the DIDS concentration corresponding to half maximal percent change under this set of experimental conditions, $K_a = 247 \pm 104$ μM and $c = 94\%$ ($r^2 = 0.88$).

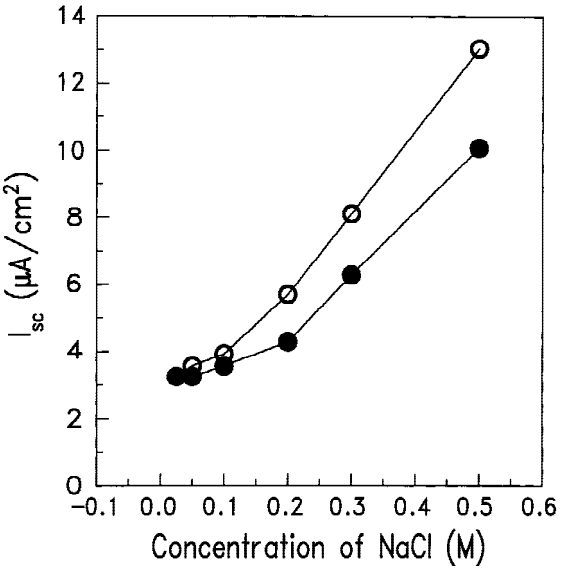

Fig. 8. Steady-state values of I_{sc} over a concentration range from 0.01 to 0.5 M NaCl in the mucosal solution, before (●) and after (○) addition of DIDS to the serosal solution in a typical experiment. DIDS enhanced I_{sc} more at higher NaCl concentrations.

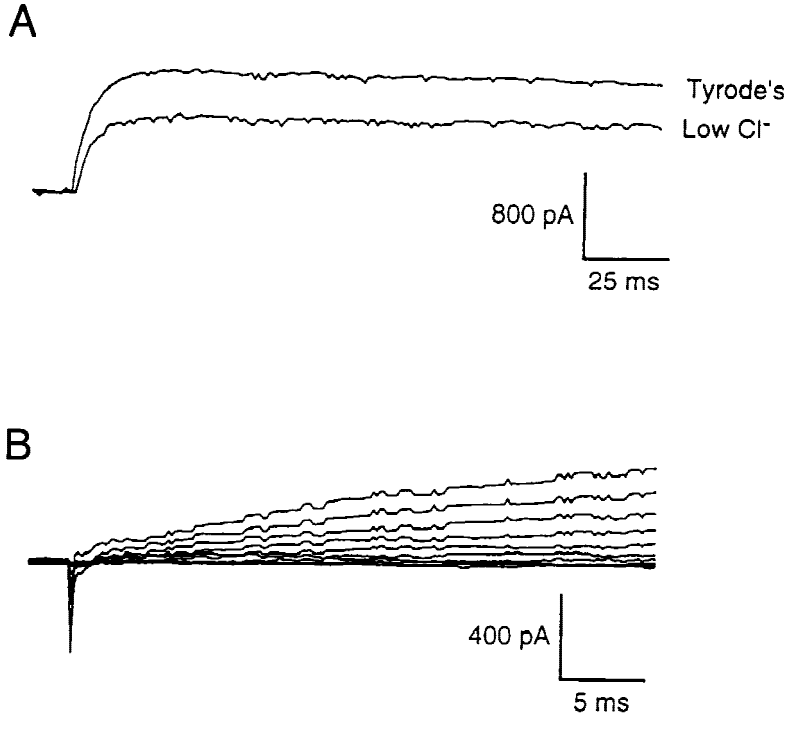

Fig. 9. Outward whole-cell current in rat fungiform taste cells. (*A*) Outward current was induced by a 175 msec voltage step to +80 mV from a holding potential of −80 mV. The top trace was recorded in normal Tyrode's (Cl^- = 151 mM; $E_{Cl} = 0$ mV) while the bottom trace was recorded in low Cl^- Tyrode's (Cl^- = 11 mM; $E_{Cl} = 66$ mV). The pipette contained standard KCl intracellular solution. (*B*) Currents elicited by a series of 35 msec depolarizing voltage pulses from a holding potential of −80 mV. The pipette contained low K^+/Cl^- intracellular solution ($E_{Cl} = -58$ mV).

similar, though smaller effects than did NFA in some cells. However, the response to FFA was less consistent than was the response to NFA, so NFA was used for most of the patch-clamp experiments.

Niflumic acid mimicked the effect of replacing the bath solution with a low Cl^- Tyrode's solution (*data not shown*); however, the effect of low bath Cl^- was always greater than the effect of NFA. When NFA was applied

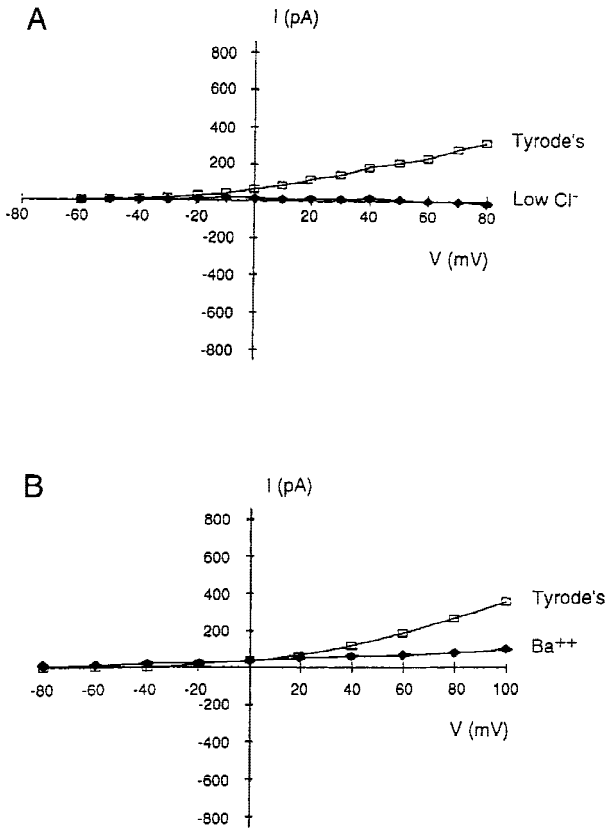

Fig. 10. Outward current has a Ca^{2+}-dependent Cl^- component. (*A*) Current-voltage relationship using the same recording conditions as for the cell shown in Fig. 9*B*. Note that the outward current is abolished when the bath Cl^- is reduced (Cl^- = 11 mM; E_{Cl} = 7 mV). (*B*) Current-voltage relationship for the cell shown in Fig. 9*B*. Note that replacement of Ca^{2+} with Ba^{2+} nearly abolished the outward current. Leak currents have been subtracted in all recordings.

to taste cells in low Cl^- Tyrode's (Cl^- = 21 mM), the effect of NFA was reduced (Fig. 11, bottom trace). Niflumic acid had no effect on membrane current in 7 taste cells, and in 4 cells niflumic acid had the opposite effect; i.e., inward current and membrane conductance were increased by bath application of NFA. These data indicate that NFA may activate a conductance in addition to blocking the Ca^{2+}-dependent Cl^- conductance. This may explain why NFA was less effective than low Cl^- and Ba^{2+} in blocking the Cl^- conductance in taste cells. In addition, NFA may not completely block the Cl^- conductance.

The relative effectiveness of NFA and FFA differed between the Ussing chamber and patch-clamp experiments. To insure that the difference was not due simply to the use of different enzymes in the tissue preparation, we repeated a few Ussing chamber experiments using the enzymes that were used to prepare the isolated taste buds for the patch-clamp experiments. Regardless of which enzymes were used to prepare the tissue, FFA always produced a higher percent inhibition of I_{sc} than did NFA. We also tested whether the difference might be due to differences in the bathing solutions (Tyrode's for taste cells and KH for Ussing measurements). The largest difference between KH and Tyrode's is the presence of HCO_3^- in the KH solution. In some whole-cell experiments, we replaced 10 mM NaCl with $NaHCO_3$. We observed no difference in the efficacy of Cl^- channel blockers under these two conditions.

Sun and Herness (1995) reported an effect of DIDS in taste cells from rat circumvallate and foliate papillae. In our whole-cell patch-clamp experiments, DIDS produced inconsistent effects (*data not shown*). In some cells DIDS increased the holding current and membrane conductance, while in other cells DIDS decreased the holding current and membrane conductance. These data suggest that DIDS may exert multiple effects on taste cells.

Discussion

The results of the Ussing chamber experiments using FFA and NFA suggest the presence of a chloride conductance in the basolateral membrane of the lingual epithelium. Since the epithelium is a heterogeneous population of cells, the patch-clamp data were necessary to determine that the chloride conductance is present in taste cells. Since only 20% of the current recorded in Ussing chamber measurements represents current flow through the taste buds (Simon et al., 1993), we cannot be absolutely sure that the conductance on the taste cells is basolateral. Nonetheless, one would expect that there would be some effect of the Cl^- channel blockers applied to the mucosal surface if an apical conductance were present on the taste cells. In addition, afferent nerve data suggest that Cl^- channel blockers applied to the surface of the tongue have no effect on Cl^- transport in taste cells (Elliot & Simon, 1990).

The patch-clamp data indicate that rat taste cells express a Ca^{2+}-dependent Cl^- conductance in most cells. The data obtained with voltage-activated currents (Fig. 9) agree with the data obtained with steady-state measurements (Fig. 11), indicating that approximately 70% of the taste cells express this conductance. The conductance appears to be blocked by NFA, although the additional presence of an NFA-activated conductance in a small subset of cells confounds the interpretation of the NFA data, since this conductance would oppose the effects of NFA on the Cl^- conductance.

In the Ussing chamber experiments, the effects of both inhibitors on transepithelial resistance are small or nonexistent relative to the effects on I_{sc}. This is probably because the net resistance is dominated by a low-resistance paracellular shunt (DeSimone et al., 1984).

The K_i values for FFA and NFA determined in the

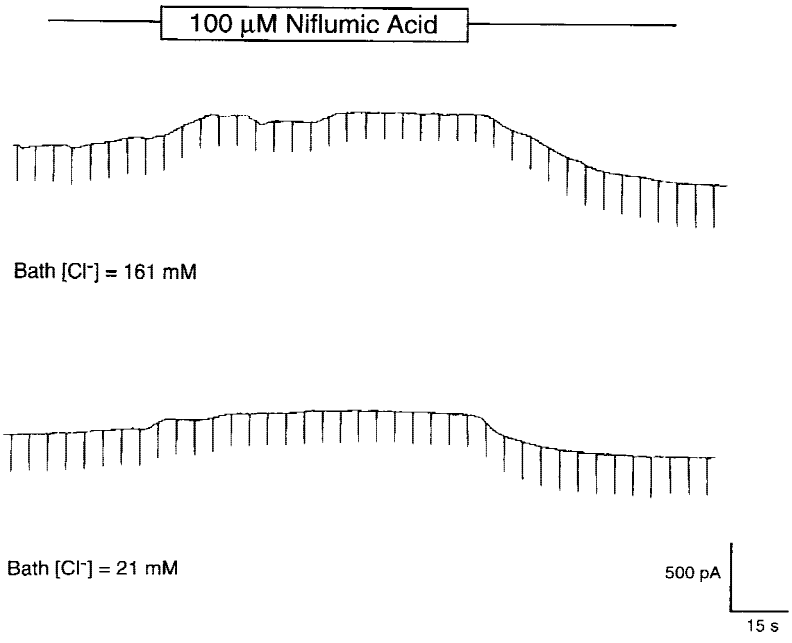

Fig. 11. Effects of niflumic acid on resting membrane conductance and holding current. The patch pipette contained low Cl^- intracellular solution. The cell was held at −80 and 20 mV hyperpolarizing voltage pulses were applied to the pipette at 5 sec intervals to assess membrane conductance. The upper trace was obtained when the cell was bathed in normal Tyrode's (161 mM Cl^-; E_{Cl} = −57 mV), the lower trace in low Cl^- Tyrode's (Cl^- = 21 mM; E_{Cl} = −5 mV). The bath solutions also contained TTX, amiloride, TEA and 4-AP to block conductances other than Cl^-.

Table. K_i values for action of FFA or NFA on Ca^{2+}-dependent Cl^- conductance in various tissues

Species and tissue	K_i for NFA (μM)	K_i for FFA (μM)	Reference
Rat brain protein expressed in *Xenopus* oocytes	17	28	White & Aylwin, 1990
Dog and cow tracheal epithelia	22	60	Chao & Mochizuki, 1992
Amphibian olfactory cilia	44	108	Kleene, 1993
Rat tongue epithelium	324	69	This study

K_i is the concentration of inhibitor that produces one half maximal inhibition.

Ussing chamber experiments indicate that in the intact epithelium FFA has a higher affinity for the chloride conductance than does NFA. The value of the K_i for FFA for the chloride conductance characterized here is similar to that found in other tissues. However, the K_i for NFA is much higher here than in other tissues (Table). In symmetrical KH, the percent inhibition of I_{sc} due to FFA was higher than for NFA at the same concentration. In patch-clamp experiments, in contrast to Ussing chamber experiments, NFA was more effective in blocking the chloride conductance than was FFA. Since the Ussing chamber measurements are dominated by current flow through the epithelial cells, it is likely that the discrepancy between Ussing chamber and patch-clamp experiments is due to different populations of Cl^- conductances in taste cells as compared to surrounding epithelial cells. It is likely that the conductance in taste cells is more sensitive to NFA, while the conductance in epithelial cells is more sensitive to FFA. It does not appear that the differences were due to either preparation of the tissue or differences in bathing solutions, although we cannot rule out these possibilities completely. The picture is confounded by the possibility that these inhibitors may affect more than one Cl^- transport process, as seems indicated for NFA from the patch-clamp data.

DIDS appears to have different effects from those of NFA or FFA. The effects differ in two ways. First, DIDS increased I_{sc} whereas NFA and FFA decreased I_{sc} (Fig. 2). Substituting gluconate for Cl^- in the serosal solution mimicked the effects of FFA and NFA but not of DIDS. Second, the concentration response function with respect to NaCl was different for DIDS than it was for either FFA or NFA (Figs. 5, 6, and 8). DIDS had no effect on I_{sc} at NaCl concentration 0.1 M and below. In contrast, NFA or FFA affected I_{sc} at all NaCl concentrations. For these reasons, it is assumed that DIDS affects a different transport process than does FFA or NFA, and hence it probably does not affect a chloride conductance. DIDS and SITS have been shown to also affect both Cl^-/HCO_3^- exchangers and non-selective cation channels (Restrepo et al., 1991; Gögelein & Pfannmüller, 1989). More experiments need to be conducted to characterize this transport process and to confirm that it is present specifically in fungiform taste cells.

Taylor and Roper (1994) identified a calcium-dependent chloride conductance in the taste cells of *Necturus* in whole-cell patch-clamp recordings; the conductance was blocked by DIDS or SITS. However, they did not use NFA or FFA. In our Ussing chamber experiments, DIDS and SITS did not have the same effect as did NFA and FFA. Therefore, it is unlikely that the Cl^-

channel in *Necturus* taste cells is the same channel that is present in rat lingual epithelium. The calcium-dependent Cl^- conductance in *Necturus* has been hypothesized to play a role in adaptation following the response to a taste stimulus (Taylor & Roper, 1994). Normally, the resting potential for most cells is close to the chloride equilibrium potential. In the presence of a mucosal hyperosmotic Cl^- solution, Cl^- could accumulate in the interstitial spaces of the taste bud, particularly since Cl^- is likely to penetrate the tight junctions at the apex of the bud (Elliot & Simon, 1990; Ye et al., 1991). This would enhance Cl^- influx through the basolateral conductance and make it less likely for the cell to fire action potentials. In addition, Ca^{2+} influx during the action potential would activate the Cl^- conductance, since it is Ca^{2+}-dependent. Indeed, large Ca^{2+}-dependent after potentials are often observed in *Necturus* taste cells (Kinnamon & Roper, 1987; Roper & McBride, 1989). Because of this, Taylor and Roper (1994) hypothesized that the chloride channel may be important for adaptation. Further studies will be required to determine if the chloride conductance found in rat taste cells plays a similar role.

It is possible that the Cl^- conductance plays a direct role in taste transduction. In taste cells in the bullfrog, removal of Cl^- from interstitial fluid in vivo affected the receptor potential induced by quinine (Okada, Miyamoto & Sato, 1988), galactose (Okada et al., 1992), or deionized water (Okada et al., 1993); it did not affect the receptor potential induced by acid stimuli (Miyamoto, Okada & Sato, 1988) or the resting potential (Sato, Okada & Miyamoto, 1995). Further studies will be required to determine if the Cl^- conductance plays a role in taste transduction in rat taste cells.

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