J. Membrane Biol. 124, 85–93 (1991)
002226319100134N

The Journal of
Membrane Biology

Ca^{2+}-Dependent Chloride Conductance in *Necturus* Taste Cells

D.W. McBride, Jr.*, and S.D. Roper
Department of Anatomy and Neurobiology, Colorado State University, Ft. Collins, Colorado 80523, and The Rocky Mountain Taste and Smell Center, University of Colorado Health Sciences Center, Denver, Colorado 80262

Summary. This report describes the occurrence and localization of a Ca^{2+}-dependent chloride conductance in taste cells of *Necturus maculosus*. Lingual epithelium from *Necturus* was removed with blunt dissection and mounted in a modified Ussing chamber which allowed individual taste cells to be impaled with intracellular micropipettes. Solutions in the mucosal and serosal chambers could be changed independently and the properties of apical and basolateral membranes tested separately. Action potentials in taste cells, elicited by brief depolarizing current pulses passed through the intracellular recording microelectrode, provided an accurate description of whether voltage-dependent conductances had been blocked or unmasked by the experimental conditions. We found that Ca^{2+} influx during the action potential triggers a prolonged depolarization due to Ca^{2+}-dependent conductance changes, particularly in the presence of TEA to block repolarizing K^+ currents. This afterdepolarization could last up to 7 sec and is due, in part, to a Ca^{2+}-dependent Cl^- conductance. Other Ca^{2+}-dependent channels such as Ca^{2+}-dependent K^+ channels or nonselective cation channels may also contribute to the afterpotential. Calcium-dependent conductance channels were situated on apical and basolateral membranes of the taste cells. We speculate that Ca^{2+}-dependent Cl^- channels may play a role in discriminating chloride salts from salts of other anions and may help shape receptor cell responses elicited by taste stimuli.

Key Words taste · chemosensory · calcium · chloride · mudpuppies · membrane conductance

Introduction

The distribution of ion channels on the apical *versus* the basolateral surface of taste cells is an important consideration in taste transduction. Chemosensory stimuli act initially on the apical surface of taste receptor cells. Conductance and membrane potential changes at the apical region then produce membrane potential changes across the basolateral surface where synapses are situated. Studies have shown that voltage-dependent K^+ channels are preferentially located on the chemosensitive, apical region of taste cells and voltage-dependent Na^+ and Ca^{2+} channels are distributed over the entire surface (Kinnamon, Dionne & Beam, 1988; Roper & McBride, 1989). Ozeki (1971) and more recent studies (Kinnamon et al., 1988; Kinnamon & Roper, 1988*a*; Spielman et al., 1989; Behe et al., 1990; Bigiani & Roper, 1991) have shown that reduction of potassium conductance may be important in the initial events in chemotransduction for a variety of taste stimuli, including sweet, sour, and bitter. Roper and McBride (1989) also describe a passive (leak) K^+ channel on the basolateral membrane. There is another class of ion channels of potential importance in chemosensory transduction, namely Ca^{2+}-dependent channels (Kinnamon & Roper, 1987). Until now, little is known about the occurrence and distribution of Ca^{2+}-dependent conductances in taste cells and their role in taste.

We have taken advantage of the fact that taste cells share many of the properties of the epithelial tissue that surrounds them. The most striking is that taste cells have a morphological polarity: taste cells possess distinct apical and basolateral membrane surfaces. Junctional complexes provide a seal between the apical and basolateral membranes and prevent many substances on one side from reaching the other surface (Holland, Zampighi & Simon, 1991). These two membrane regions can be studied separately by mounting isolated sheets of lingual epithelium in a modified Ussing chamber (*cf.* Roper & McBride, 1989) and in this way the properties of apical and basolateral ion channels can be investigated by ion substitution experiments or by pharmacological manipulations.

Kinnamon and Roper (1988*b*) found that a Ca^{2+}-dependent K^+ conductance produced a long afterpotential following impulses in taste cells. However, the Ca^{2+}-dependent K^+ conductance did not fully explain the observations and the data indicated

* *Present address:* Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

that other ions were involved. This report describes a Ca^{2+}-dependent Cl^- conductance in taste cells. The data suggest the channels are situated on both apical and basolateral membranes, in contrast with the apically-situated delayed rectifier potassium channels.

Materials and Methods

Mudpuppies (*Necturus maculosus*) were obtained from commerical suppliers and maintained at 4–10°C. in aquaria containing 2% artificial seawater. Mudpuppies were fed earthworms and minnows.

Ussing Chamber

Lingual epithelium dissected from *Necturus* was mounted in a modified Ussing chamber, and individual taste cells were impaled with glass micropipettes as described previously (Roper & McBride, 1989). Briefly, after the lingual epithelium was removed from the tongue with blunt dissection, the tissue was mounted in a specially constructed Ussing chamber that was initially filled with amphibian physiological saline (APS). The tissue was mounted horizontally and the Ussing chamber was placed under a compound microscope so that the tissue could be viewed with a water immersion 40× objective using Nomarski differential interference contrast optics. In this way, the mucosal surface of the lingual epithelium could be scanned, taste buds and individual cells identified, and cells impaled with glass microelectrodes.

Solutions

Our standard APS consisted of 112 mM NaCl; 2 mM KCl; 8 mM $CaCl_2$; 5 mM HEPES buffer (pH 7.2). Calcium chloride was elevated to 8 mM to improve the stability of the microelectrode impalements. Other solutions were made by ion substitutions. When ion substitutions were made, as described in the text, care was taken to maintain isotonicity (252 mOsM).

Electrical Recording

Micropipettes for intracellular recording were filled with 2.5 M KCl and had resistances of 80–200 MΩ. However, in many of the experiments described in the present report, intracellular recording micropipettes were filled with 4 M K-acetate rather than 2.5 M KCl. This was done to prevent loading the taste cell with Cl^-, as described in the text below. Microelectrodes were inserted into taste cells under visual control at 400×. The reference electrode generally was immersed in the serosal chamber, although using the mucosal chamber as a reference did not alter the findings. A bridge circuit (W. P. Instruments) was used to pass current into the cell and record the membrane potential simultaneously from a single microelectrode. Stable impalements with resting potentials exceeding −50 mV and input resistances over 200 MΩ could often be maintained for long periods (>90 min) while continually perfusing both compartments of the Ussing chamber.

Experimental Protocol

Our standard protocol consisted of bathing the apical and basolateral surfaces of the epithelium initially in symmetrical solutions of APS. The microelectrode was positioned near a taste bud and inserted into a taste cell until a stable impalement was achieved. The chief criterion for identifying a taste cell was the presence of action potentials in response to brief depolarizing current pulses (Roper, 1983). After a period of about 5 min for stabilization, the solutions bathing one or both of the chambers were exchanged from APS to experimental media. The time constant for solution change for the top (mucosal, apical) and bottom (serosal, basolateral) chambers was about 1–5 min. Test solutions were replaced with APS at the end of the experiment and control recordings rechecked for stability.

Voltage-dependent ion conductances were studied as described previously (Roper & McBride, 1989). Namely, the taste cell action potential and the changes in its amplitude and duration in response to pharmacological agents and/or ion substitutions provided an accurate description of whether one or more voltage-dependent conductances had been blocked and/or other conductances revealed by the experimental procedures.

Results

It is by now well established that taste cells are capable of producing regenerative impulses (Kashiwayanagi, Miyake & Kurihara, 1983; Roper, 1983; Avenet & Lindemann, 1987). In *Necturus*, when a taste cell produces a regenerative action potential, the initial inward current includes a significant Ca^{2+} contribution (Kinnamon & Roper, 1988*b*; Kinnamon, Roper & Beam, 1989). These findings have been confirmed and extended in taste cells from salamanders (Sugimoto & Teeter, 1990) and rats (Behe et al., 1990). The contribution of Ca^{2+} current appears as a slight plateau on the action potential (Roper, 1983). The influx of Ca^{2+} often triggers a prolonged Ca^{2+}-dependent conductance change which produces a late afterpolarization following the impulse (Kinnamon & Roper, 1987). The following tests were conducted to examine this Ca^{2+}-dependent conductance in greater detail.

The amplitude of the prolonged afterpotential which follows the impulse was quite variable from cell to cell and depended strongly upon the resting potential of the taste cell. To establish more uniform conditions for comparing responses from one cell to the next, the following standard condition was established: we set the resting potential at −100 mV just prior to recording any responses by passing a small constant current through the intracellular microelectrode after balancing the bridge circuit. In the absence of this dc current, resting potentials recorded in symmetrical solutions of APS (i.e., identical medium in the mucosal and serosal chambers) usually varied from −60 to −80 mV and some-

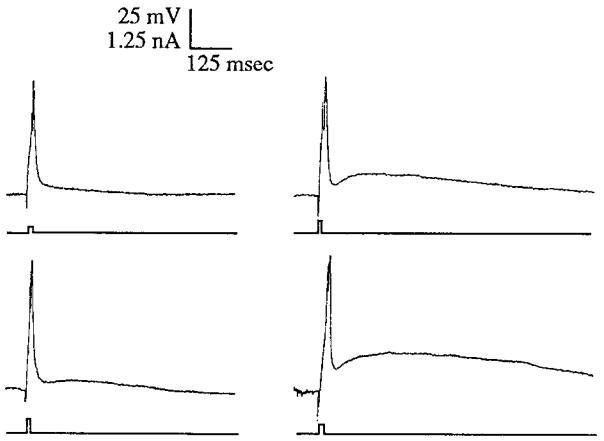

Fig. 1. Examples of action potentials recorded intracellularly from *Necturus* taste cells. Action potentials were evoked by passing a brief depolarizing current pulse (lower traces) through the intracellular microelectrode. Note the prolonged afterpotential seen in each example. In this and all subsequent figures, the resting potential was −100 mV, established by passing a small hyperpolarizing dc current through the intracellular microelectrode, as described in Materials and Methods. Calibration shows mV for upper trace and nA for lower trace in each pair of records.

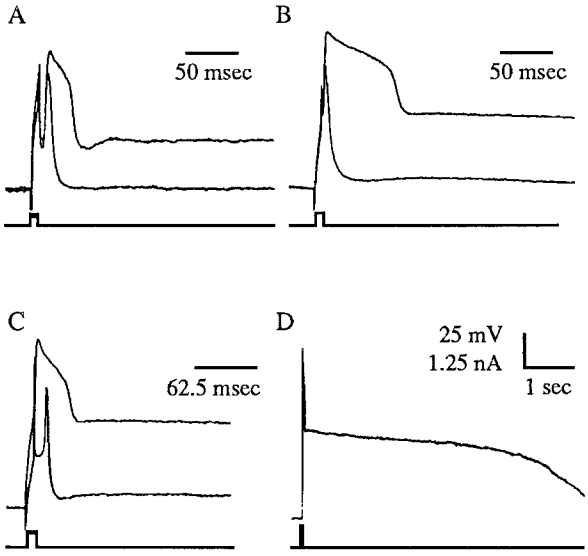

Fig. 2. Action potentials in *Necturus* taste cells recorded before and after adding TEA to the mucosal (apical) bath. Action potentials were evoked by passing brief current pulses through the intracellular recording microelectrode (bottom traces in each record). (*A–C*) Two intracellular recordings are superimposed in each panel. Lower trace in each pair of the intracellular recordings is prior to adding TEA, and uppermost trace, after adding 8 mM TEA to the mucosal bath. (*D*) An example of an exceptionally prolonged afterpotential in the presence of TEA. The voltage and current calibration in *D* applies to *A–C* as well.

times as high as −100 mV. Even with the membrane potential set at a constant value of −100 mV, the afterpolarization was still quite variable, possibly reflecting differences in either the Ca^{2+} current and/or Ca^{2+}-dependent currents from cell to cell (*cf.* Kinnamon & Roper, 1988*b*) or in the quality of the microelectrode impalement. Figure 1 illustrates the variability in the afterpolarization in typical recordings from four different taste cells bathed in symmetrical solutions of APS and with the initial membrane potential set to −100 mV.

The afterpolarization could be greatly enhanced by blocking repolarizing outward current through voltage-dependent K^+ channels (delayed rectifier channels) that are situated in the apical membrane. This unmasked a prolonged depolarization. Figure 2 shows records from four different taste cells in which TEA was present in the medium bathing the *apical* membrane. Under these conditions, voltage-dependent inward Na^+ and inward Ca^{2+} currents are unaffected, but outward K^+ current is inhibited (Kinnamon & Roper, 1988*b*) and Ca^{2+}-dependent K^+ channels are blocked (Blatz & Magleby, 1987; Kolb, 1990). In the presence of TEA, action potentials were increased in amplitude and lengthened and afterdepolarizations were strikingly enhanced. The afterdepolarization varied in amplitude and duration from cell to cell. For example, in some cases, the after depolarization lasted up to 7 sec before the membrane repolarized to the resting potential (Fig. 2*D*).

When only the *basolateral* membrane of taste cells were exposed to TEA, thereby avoiding blocking the delayed rectifier in the apical membrane, the afterpotentials became depolarizing responses in only about half the cases (n = 8/15 cells; Fig. 3). In no case was the afterdepolarization in the presence of basolateral TEA ever as pronounced as that produced by apical TEA application. This is consistent with our previous findings (Roper & McBride, 1989) that TEA had only a small, variable effect on potassium conductance of the basolateral membrane.[1]

Ca^{2+}-Dependent Conductances Underlie the Afterpotential

The prolonged afterdepolarization that was observed in the presence of TEA in most taste cells

[1] In the previous report we also noted that the effects of TEA on the basolateral membrane of *Necturus* taste cells were subject to seasonal variations (Roper & McBride, 1989). This was not explored in the present series of experiments on Ca^{2+}-dependent conductances.

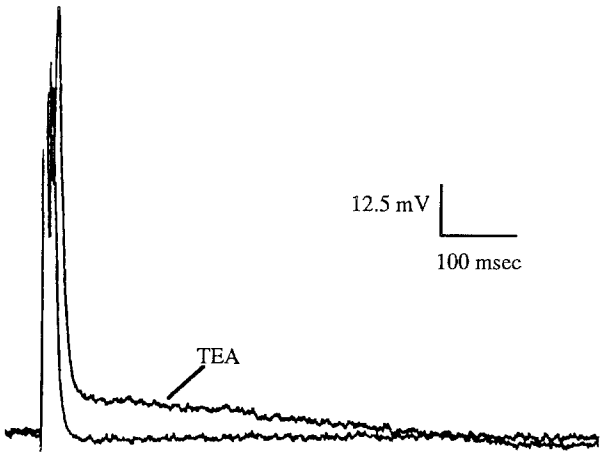

Fig. 3. Effect of TEA, applied to the basolateral membrane, on the afterpotential that follows an impulse in *Necturus* taste cells. Two responses to brief depolarizing current pulses (*not shown*) passed through the intracellular recording microelectrode are superimposed. The bottom trace is the response recorded in symmetrical solutions of APS. The trace marked *TEA* shows the response when 8 mM TEA was added to the serosal (basolateral) bath.

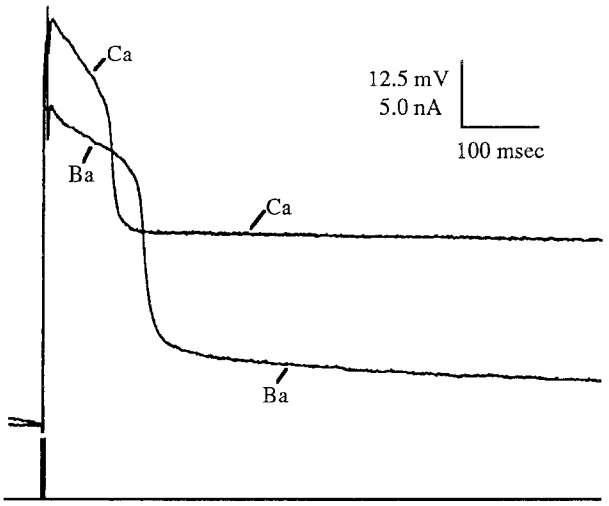

Fig. 4. The afterpotential that follows an impulse in *Necturus* taste cells is generated by calcium-dependent processes; replacing Ca^{2+} with Ba^{2+} reduces the afterdepolarization. The data show two superimposed responses elicited by brief intracellular depolarizing current pulses (current trace at bottom of records), recorded from the same cell before and after Ca^{2+} replacement. In both mucosal and serosal chambers, 8 mM TEA was included to block potassium conductance and 1 μM tetrodotoxin (TTX) was added to prevent any voltage-dependent Na^+ currents from contributing to the responses. In the trace marked *Ca*, 60 mM $CaCl_2$ was present in the mucosal and serosal baths. NaCl was reduced to 34 mM to maintain isotonicity. The trace marked *Ba* was recorded after $CaCl_2$ was omitted and 30 mM $BaCl_2$ plus 30 mM $MgCl_2$ added to the bathing media. $MgCl_2$ itself had no effect on the responses and was included only to balance the osmotic strength of the medium. NaCl concentration was unchanged (34 mM). When $[Ca]_o$ was replaced with $[Ba]_o$, the action potential was broadened, consistent with a greater influx of the more permeant divalent cation, Ba^{2+}, during the initial depolarization. Note, however, the afterpotential was greatly reduced in amplitude in the presence of Ba^{2+}.

was due to Ca^{2+}-dependent conductance changes, as will be shown below. Kinnamon and Roper (1987) had already shown that afterdepolarizations observed in *Necturus* taste cells in the absence of TEA were eliminated by 0.1 mM $CdCl_2$, a potent Ca^{2+} channel blocker in this tissue. In the present experiments, the role of Ca^{2+} in the prolonged afterpotential was tested by replacing Ca^{2+} with Ba^{2+} in the serosal and mucosal baths.[2] Figure 4 illustrates results from an experiment in which action potentials were recorded first in the presence of elevated $CaCl_2$ (60 mM) to enhance calcium influx, and then after replacing all $CaCl_2$ with 30 mM $BaCl_2$.[3] Substituting Ba^{2+} for Ca^{2+} reversibly reduced or abolished the prolonged afterdepolarization in all cases.

The afterpotential that follows the action potential in *Necturus* taste cells reflects the activation of a long-lasting conductance increase. This was established by applying a series of brief hyperpolarizing current pulses of constant amplitude during the afterpotential. Figure 5 shows the results of one such experiment where the resting input resistance decreased from 260 to 49 MΩ at the peak of the afterdepolarization.

The currents that underlie the prolonged afterpotential are not themselves markedly voltage dependent. That is, the afterpotential is not, of itself, a maintained regenerative event, such as a prolonged regenerative Ca^{2+} current. We showed this by repolarizing the membrane during the afterpotential. Figure 6 illustrates that repolarizing the membrane back to −100 mV for up to 1.5 sec had no effect on the subsequent amplitude or duration of the afterpotential.

Ion Conductances Underlying the Afterpotential

Kinnamon and Roper (1987) discussed how Ca^{2+}-dependent K^+ conductance ($g_{K(Ca)}$) changes occur after the action potential in *Necturus* taste cells, but that $g_{K(Ca)}$ alone cannot account for the afterpolarization that follows an action potential in *Necturus*

[2] Ba^{2+} is not as effective as Ca^{2+} in activating Ca^{2+}-dependent conductances and is even known to block Ca^{2+}-dependent K^+ channels (Gorman, Woodlum & Cornwall, 1982).

[3] We replaced 60 mM $CaCl_2$ with 30 mM $BaCl_2$ since Ba^{2+} has been shown to be about twice as conductive as Ca^{2+} through Ca^{2+} channels in *Necturus* taste cells (Kinnamon & Roper, 1988*b*).

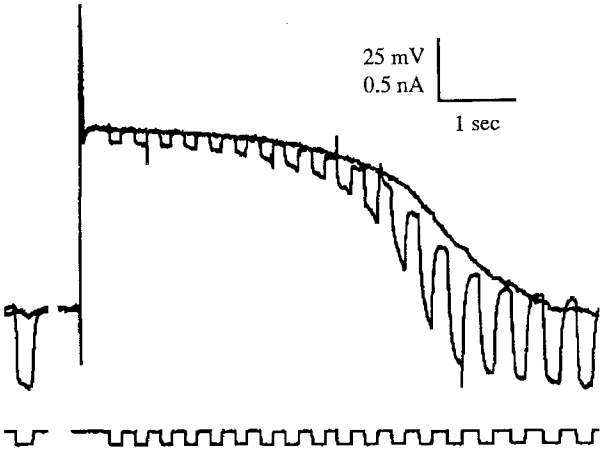

Fig. 5. The afterpotential is associated with a membrane conductance increase. Shown here is an action potential evoked by a brief depolarizing current passed through the intracellular microelectrode (*not shown*). Two consecutive records are superimposed. During one, a train of constant current hyperpolarizing pulses (bottom trace) was passed through the intracellular electrode before and during the action potential to monitor the input resistance of the taste cell. TEA (8 mM) was present in the mucosal bath.

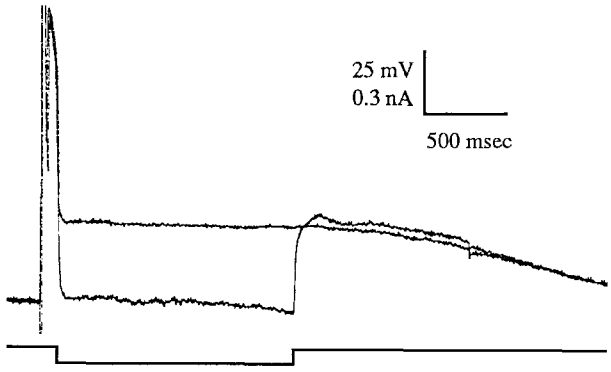

Fig. 6. Repolarizing the membrane to the resting potential during the afterpotential does not abolish the afterdepolarization. Two consecutive action potentials elicited by brief depolarizing current pulses (*not shown*) are superimposed. After the second action potential, a 1.5-sec hyperpolarizing pulse was injected (bottom trace) to bring the membrane back to the initial membrane potential (−100 mV) during a portion of the afterpotential. This did not inactivate the afterpotential, which returned to its former level as soon as the hyperpolarizing step was removed. TEA (8 mM) was present in the mucosal bath.

taste cells. Calcium-activated Cl^- conductances ($g_{Cl(Ca)}$) have been shown to occur in other tissues, including sensory receptors (e.g., Bader, Bertrand & Schwartz, 1982; *cf.* review by Marty, 1989). Thus, we next tested whether the Ca^{2+}-activated afterpotential was elicited by an increased Cl^- conductance. The following experiments indicated that a substantial portion of the afterpotential was indeed caused by a Ca^{2+}-dependent Cl^- current.

Initially, we attempted to reduce extracellular Cl^- to 10 mM with isethionate substitution and determine how this affected the amplitude of the afterpotential. These experiments were not particularly satisfactory. Completely exchanging Cl^- in the basolateral extracellular spaces surrounding taste cells was too slow a process to maintain an impalement for the duration. Furthermore, long periods (>1 hr) in isethionate salts seemed to have deleterious effects on the tissue: among other observations, we noticed that the duration of the action potential was often reduced, thereby potentially decreasing the influx of Ca^{2+} and interfering with Ca^{2+}-activated processes. Lastly and most importantly, even though extracellular Cl^- could eventually be reduced to 10 mM, it is likely that, with time, intracellular Cl^- equilibrates passively with little net change in the Cl^- gradient across the membrane (i.e., E_{Cl} would be relatively unchanged).

A more conclusive test was to change E_{Cl} by manipulating *intracellular* Cl^-. To achieve this, we impaled taste cells with *KCl*-filled micropipettes and compared the findings with experiments when cells were impaled with K^+ *acetate*-filled micropipettes. Calculations (below) indicated that impaling taste cells with KCl-filled micropipettes was a quick and effective method of raising $[Cl^-]_i$ significantly. Afterpotentials were markedly depolarizing in those cells impaled with KCl-filled pipettes compared with responses recorded with K^+ acetate-filled pipettes. Figure 7 illustrates afterpotentials in taste cells when recorded with KCl-filled and K^+ acetate-filled micropipettes. The Table summarizes these data.

If the afterpotential was solely due to Cl^- fluxes in the presence of TEA to block $g_{K(Ca)}$, the depolarization recorded with KCl-filled microelectrodes (mean = −41 mV; Table) would indicate an intracellular Cl^- concentration of about 26 mM. This would correspond to a two- to fivefold increase in the total resting intracellular Cl^- (resting $[Cl]_i$ was estimated at 5–12 mM[4]). This elevated value could quickly be reached after impaling a cell with a 100-MΩ KCL-filled micropipette.[5]

[4] Resting $[Cl]_i$ was calculated assuming Cl^- is distributed passively at rest (i.e., $E_{Cl} = V_m$) and on the basis of the observed resting potentials under these experimental conditions, namely between −60 to −80 mV.

[5] Calculations have been made of the expected efflux of salts from micropipette tips (*cf.* Coombs, Eccles & Fatt, 1955), and these effluxes have been verified experimentally (e.g., Fromm & Schultz, 1981). Based upon these calculations, we would anticipate that a theoretical maximum spontaneous efflux of Cl^- from the KCL-filled microelectrodes employed in the present experiments (average = 100 MΩ resistance) would be approximately 1.3 feq/sec. Since a background current of about

Table. Comparison of afterpotentials in experiments in which $[Cl]_i$ in taste cells had been manipulated by utilizing micropipettes filled with KCl or filled with K-acetate[a]

	KCl-filled pipettes	K actetate-filled pipettes
Afterpotential	−41 ± 19 mV (19)	−79 ± 14 mV (10)
Resting R_i	317 ± 135 MΩ (8)	372 ± 325 MΩ (6)
R_i during afterpotential	114 ± 59 MΩ (8)	313 ± 284 MΩ (6)
Decrease in R_i during afterpotential	64%	16%

[a] TEA (10 mM) was present in the mucosal bath to block repolarizing K currents. The resting potential was set to −100 mV for each cell, as described in Materials and Methods. R_i = input resistance, measured by passing small hyperpolarizing pulses through the intracellular micropipette and recording the resultant membrane potential deflections. Resting R_i = input resistance measured at −100 mV. Data in parentheses refer to the numbers of taste cells in each sample. Values are given ± SD. The amplitude of the afterpotential and R_i during the afterpotential were measured immediately after an action potential when the afterdepolarization reached an initial plateau. The amplitude of the afterpotential is given in absolute membrane potential, not as a difference from rest.

These data indicate that a large part of the Ca^{2+}-dependent conductance change which follows action potentials in *Necturus* taste cells is due to a long-lasting Cl^- conductance increase. In three cases, 2 mM furosemide was added to the mucosal and serosal baths to determine whether the prolonged afterpotential was affected by this pharmacological agent, which is known to block Ca^{2+}-dependent Cl^- conductances in other tissues (L'Allemain, Paris & Pouyssegur, 1985). Furosemide had no or little effect in our experiments, indicating that the pharmacology of these Cl^- channels may not be the same as those previously described in other cells.

We also conducted ion substitution experiments to determine if other ions, especially Na^+, contribute to the long-lasting afterpotential. We eliminated all voltage-activated and Ca^{2+}-dependent currents across the apical membrane of the taste cells: Ca^{2+} and Na^+ were replaced with the impermeant ion, N-methyl D-glucamine ($NMDG^+$) in the mucosal bath, and 8 mM TEA was added to eliminate K^+ currents. At the beginning of the experiment, the serosal bath contained 30 mM $CaCl_2$ (to enhance Ca^+ entry across the basolateral membrane during action potentials), and 79 mM NaCl (to balance the osmolarity). KCl and HEPES were the same as in standard APS. In some experiments, 10 μM tetrodotoxin (TTX) was added to mucosal and serosal baths to eliminate voltage-dependent Na^+ currents, too, but the data from these experiments did not differ from those in which TTX was not included. Taste cells generated Ca^{2+} action potentials under these conditions and long-duration afterpotentials were still elicited (Fig. 8*A*, upper trace). Next, all Na^+ in the serosal bath was replaced by an equivalent amount of $NMDG^+$ and the action potentials recorded in this ionic environment (Fig. 8*A*,

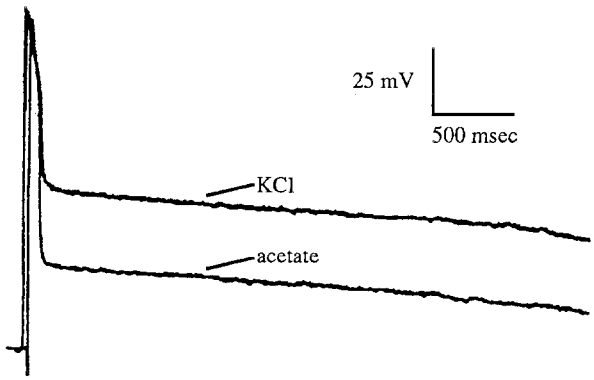

Fig. 7. The effect of raising intracellular $[Cl^-]$ on the afterpotential that follows an impulse in *Necturus* taste cells. Recordings from two separate experiments are superimposed. TEA (8 mM) was present in the mucosal bath. The trace labeled *acetate* illustrates an action potential elicited by a brief pulse of depolarizing current (*not shown*) recorded with a K^+ acetate-filled microelectrode. In this case $[Cl^-]_i$ was estimated to be 5–12 mM (*cf.* text). The trace labeled *KCl* illustrates an action potential recorded with a KCl-filled microelectrode. Under these circumstances, $[Cl^-]_i$ was 2–5 times higher as in trace labeled *acetate* due to Cl^- efflux from the intracellular microelectrode (*cf.* text).

−0.2 nA was routinely applied to set the taste cell resting potential at −100 mV, this would add another 1 feq/sec efflux of Cl^- ions. The taste cell is an elongate cell about 110 μm in length, 13 μm in diameter at its widest point and tapering to under 5 μm at either pole (Yang & Roper, 1987; Delay & Roper, 1988). The volume of the taste cell is thus between 3 to 4 pl. An influx of 2–3 feq/sec Cl^- into this volume would be approximately 50% the original intracellular Cl^- content in <24 sec! Without a knowledge of the resting transmembrane Cl^- efflux, the actual steady-state value of $[Cl^-]_i$ due to leakage from the intracellular micropipette cannot be calculated. However, a value of 26 mM (40–80 feq above the resting content, 5–12 mM) is not unreasonable.

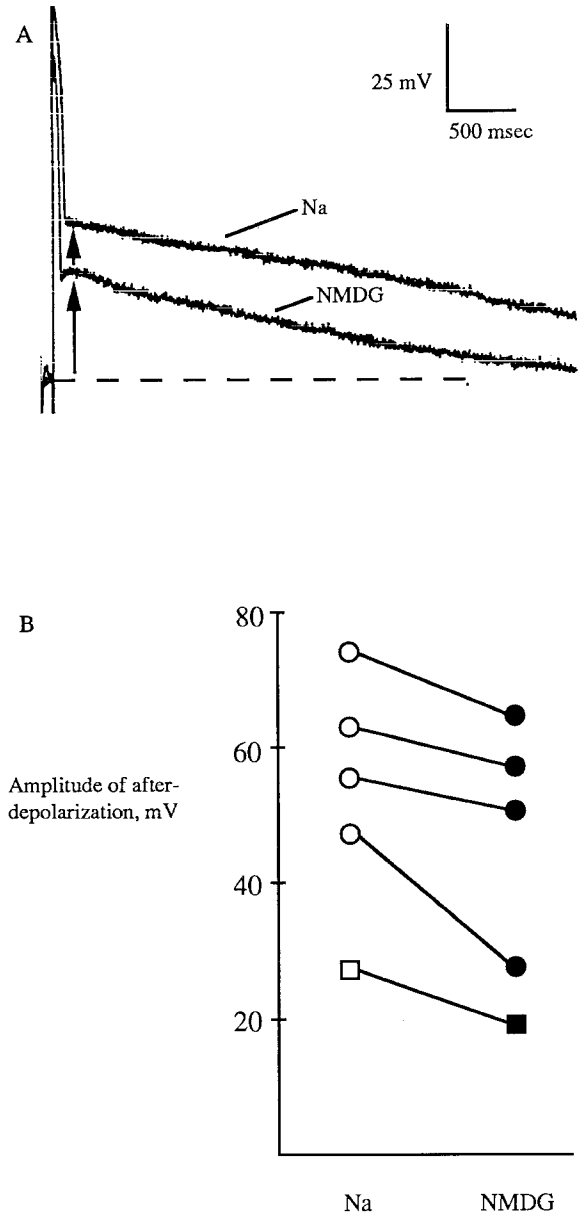

Fig. 8. Replacing serosal Na^+ with $NMDG^+$ decreases the amplitude of the afterdepolarization, suggesting that Na^+ fluxes across the basolateral membrane also contribute to the prolonged responses that follow action potentials in *Necturus* taste cells. (*A*) Two recordings from the same experiment are superimposed. The trace marked *Na* was recorded when the serosal bath containing 30 mM $CaCl_2$, 79 mM NaCl, 2 mM KCl, and 5 mM HEPES buffer. The ionic composition of the mucosal bath is described in the text. The trace labeled *NMDG* shows the action potential in same cell after replacing NaCl with 79 mM NMDG-Cl. Vertical arrows represent the amplitude of the afterdepolarization in mV above the initial membrane potential (−100 mV, dashed line). (*B*) Plot of data from five different experiments, showing amplitudes of afterpotentials (e.g. vertical arrows in *A*) in presence of 79 mM NaCl (open symbols) and after all Na^+ had been replaced by $NMDG^+$ (filled symbols). Circles show data recorded with KCl-filled micropipettes and squares show data from one experiment in which recordings were made with a K acetate-filled microelectrode.

lower trace marked *NMDG*). The amplitude of the afterpotential consistently decreased (n = 5 experiments) when Na^+ ws replaced by $NMDG^+$. These data suggest that Ca^{2+}-dependent ionic conductances that involve Na^+, such as nonselective cation channels, may also contribute to the afterpotential in taste cells, although we cannot rule out that small changes in the initial Ca^{2+} influx had occurred during NMDG treatment.

The Regional Distribution of Ca-Dependent Conductances

The focus of the remaining experiments is on the localization of the Ca^{2+}-dependent conductance channels to apical, basolateral, or both regions of the taste cell. The protocol for isolating the membrane region(s) which possess Ca^{2+}-dependent channels was to bathe the apical and basolateral surfaces of the cells in solutions of different ionic composition. To localize the Ca^{2+}-dependent channels, *both* sides of the tissue were initially bathed in a solution in which Ca^{2+} had been replaced with Ba^{2+} and TEA added to block repolarizing K^+ currents. Under these conditions, large Ba^{2+} impulses were recorded in response to brief depolarizing impulses passed through the recording microelectrode (*cf.* Fig. 4). When Ca^{2+} was reintroduced into (and Ba^+ eliminated from) the *mucosal* (apical) chamber, the afterpotential reappeared (Fig. 9*A*), suggesting that the apical membrane possesses Ca^{2+}-dependent channels (however, *cf.* Discussion). Conversely, when Ca^{2+} was reintroduced only into the *serosal* chamber, similar results were obtained (Fig. 9*B*), indicating that Ca^{2+}-dependent channels are also present in the basolateral membrane.

Discussion

The main finding of this report is that taste cells in *Necturus* possess Ca^{2+}-dependent channels on their apical and basolateral regions, and specifically a Ca^{2+}-dependent Cl^- conductance. Other Ca^{2+}-dependent channels, such as K(Ca) channels and possibly nonselective cation channels are also present in *Necturus* taste cells. Under normal physiological conditions, increases in a Cl^- conductance would not necessarily produce a change in the membrane potential since E_{Cl} is believed to be near the resting potential. However, its presence can be unmasked, as in the present experiments, by decreasing E_{Cl} and/or by blocking K^+ conductance. Under these conditions the Ca^{2+}-dependent Cl^- conductance produces a marked depolarization.

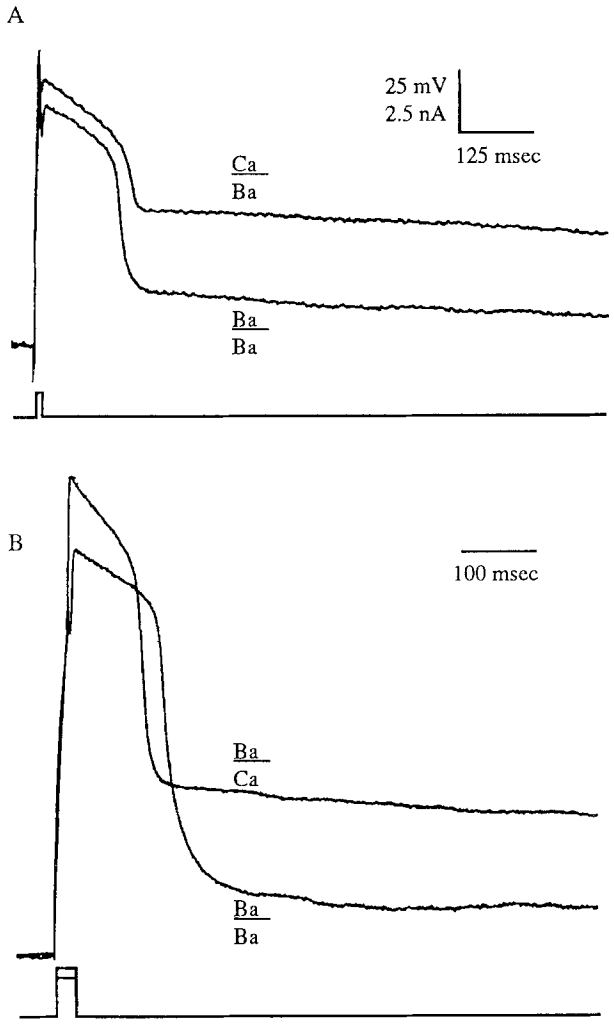

Fig. 9. Ca^{2+}-dependent conductance is a property of the apical and basolateral membranes in *Necturus* taste cells. (*A*) Action potentials and afterdepolarizations elicited by brief depolarizing current pulses (bottom trace) passed through the intracellular microelectrode. Two recordings from the same cell are superimposed. At the beginning of the experiment, action potentials were elicited in the presence of symmetrical solutions containing 0 mM $CaCl_2$, 15 mM $BaCl_2$, 15 mM $MgCl_2$ and 74 mM NaCl (trace labeled *Ba/Ba*). $MgCl_2$ itself had no effect on the responses and was included only to balance the osmotic strength. In the mucosal and serosal chambers TTX (0.5 μM) was added to eliminate voltage-dependent Na^+ currents and 8 mM TEA added to block K^+ currents. Subsequently (trace labeled *Ca/Ba*), 30 mM $CaCl_2$ was added to (and $BaCl_2$, $MgCl_2$ removed from) the mucosal (apical) chamber. All other components of the mucosal and serosal baths were left unchanged. Replacing apical Ba^{2+} with Ca^{2+} enhanced the depolarizing afterpotential. (*B*) A different taste cell with the converse protocol to the experiment illustrated in *A*. The lower trace (labeled *Ba/Ba*) shows an action potential recorded first in symmetrical bathing solutions of 30 mM $BaCl_2$. In addition, 30 mM $MgCl_2$, 34 mM NaCl, 1 μM TTX and 8 mM TEA were present in serosal and mucosal baths. The upper trace (labeled *Ba/Ca*) was recorded after 60 mM $CaCl_2$ was added back to (and $BaCl_2$, $MgCl_2$ removed from) the basolateral bathing medium. All other components of the mucosal and serosal baths were left unchanged. In both *A* and *B*, restoring Ca^{2+} either to the apical or to the basolateral bathing solutions resulted in a recovery of the depolarizing afterpotential.

Calcium fluxes may play an important role in taste transduction. Specifically, action potentials elicited by chemical stimulation will cause an influx of Ca^{2+}. This influx, plus any Ca^{2+} released from internal stores during chemostimulation (Akabas, Dodd & Al-Awqati, 1988), would be expected to have two effects: (i) it would cause neurotransmitter release at receptor cell synapses; and (ii) it would open Cl^-, K^+, and possibly other Ca^{2+}-dependent channels in the apical and basolateral membranes. The action of the Ca^{2+}-dependent conductances in taste cells could be to repolarize the membrane after impulses, thereby terminating or reducing any maintained excitatory receptor currents. Thus, Ca^{2+}-dependent Cl^- and K^+ channels might serve as inhibitory feedback mechanisms in chemosensory transduction and thereby modify responses to taste stimuli.

Our findings on the presence of a long-duration Ca^{2+}-dependent chloride conductance in taste cells suggests a mechanism for how anions might contribute to taste discrimination. That is, although cations play a dominant role in salt taste transduction, the anion is also known to influence the taste quality (Schiffman, McElroy & Erickson, 1980). Since taste stimulation may activate a long-lasting Cl^- conductance under certain conditions, as described in this report, the membrane potential of the taste cell may be strongly influenced by the Cl^- gradient across the apical membrane. For example, an elevated $[Cl]_o$ during NaCl stimulation would shift E_{Cl} to more negative values. Any increase in g_{Cl} during NaCl stimulation would enhance Cl^- influx. This would oppose the depolarizing current generated by Na^+ influx if g_{Na} and g_{Cl} occurred on the same receptor cell. If, on the other hand, taste cells were exposed to sodium salts consisting of anions that do not permeate chloride channels, this would shift E_{Cl} to more depolarized potentials. Activation of Ca^{2+}-dependent Cl^- conductances under these conditions might even lead to a frank efflux of Cl^-, i.e., a depolarizing current. It is not yet possible to analyze the membrane potential changes in detail since one would need to know the relative contributions of the Ca^{2+}-dependent Cl^- conductances on the apical *versus* basolateral surfaces, the precise ionic gradients across these two surfaces during salt stimulation, and the relative permeability of chloride channels to ions other than Cl^-, among other factors. Whether calcium-dependent Cl^- conductance increases result in prolonged depolarizations or hyperpolarizations during taste stimulation, and how this might be translated into different taste qualities among salts remains to be determined. Other mechanisms that involve differences in paracellular epithelial shunt resistances have also been proposed to explain how anion salts are discriminated (Elliott & Simon, 1990).

A caveat needs to be added regarding our localization of the Ca^{2+}-dependent chloride conductances to the apical and basolateral membranes. Our experiments to determine this were dependent upon the fact that entry of Ca^{2+} across the membrane, either apical or basolateral, would exert its effects locally. If, on the other hand, Ca^{2+} permeated one membrane and diffused to the other, "*trans*", membrane, or caused Ca^{2+} release from intracellular stores, this would invalidate our conclusion that the Ca^{2+}-dependent conductance was localized in the first ("*cis*") membrane. We have no way to determine whether this translocation of calcium occurred.

An intriguing aspect of the Ca^{2+}-dependent Cl conductance described in this study is its long duration. An influx of Ca^{2+} during the action potential lasts only a few msec, yet the afterpotential can last for several seconds. The prolonged duration of the increased Cl_{Ca} conductance suggests that some, as yet unexplored, cytosolic changes subsequent to Ca^{2+} entry, for example involving second messengers, may open Cl channels or may prevent them from closing, once open. cAMP-dependent Cl conductance that is mediated by increased intracellular Ca^{2+} is known to occur in other epithelia (*cf.* Frizzell, Rechkemmer & Shoemaker, 1986) and similar mechanisms may operate in taste cells.

We would like to thank Drs. P. Avenet, A. Bigiani, D. Ewald, S. Kinnamon and A. Mackay-Sim for a critical reading of this manuscript. The work was funded by NIH grants DC00374 and DC00244.

References

Akabas, M., Dodd, J., Al-Awqati, Q. 1988. A bitter substance induces rise in inracellular calcium in a subpopulation of rat taste cells. *Science* **242:**1047–1050

Avenet, P., Lindemann, B. 1987. Action potentials in epithelial taste receptor cells induced by mucosal calcium. *J. Membrane Biol.* **95:**265–269

Bader, C.R., Bertrand, D., Schwartz, E.A. 1982. Voltage-activated and calcium-activated currents studied in solitary rod inner segments from the salamander retina. *J. Physiol.* **331:**253–284

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

Bigiani, A., Roper, S.D. 1991. Mediation of responses to calcium in taste cells by modulation of a potassium conductance. *Science* **252:**126–128

Blatz, A.L., Magleby, K.L. 1987. Calcium-activated potassium channels. *Trends Neurosci.* **10:**463–467

Coombs, J.S., Eccles, J.C., Fatt, P. 1955. The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory post-synaptic potential. *J. Physiol.* **130:**396–413

Delay, R.J., Roper, S. 1988. Ultrastructure of taste cells and synapses in the mudpuppy. *Necturus maculosus*. *J. Comp. Neurol.* **277:**268–280

Elliott, E.J., Simon, S.A. 1990. The anion in salt taste: A possible role for paracellular pathways. *Brain Res.* **535:**9–17

Frizzell, R.A., Rechkemmer, G., Shoemaker, R.L. 1986. Altered regulation of airway epithelial cell chloride channels in cystic fibrosis. *Science* **233:**558–560

Fromm, M., Schultz, S.G. 1981. Some properties of KCl-filled microelectrodes: Correlation of potassium "leakage" with tip resistance. *J. Membrane Biol.* **62:**239–244

Gorman, A.L.F., Woodlum, J.C., Cornwall, M.C. 1982. Selectivity of the Ca^{2+} activated and light-dependent K^+ channels for monovalent cations. *Biophys. J.* **38:**319–322

Holland, V.F., Zampighi, G.A., Simon, S.A. 1991. Tight junctions in taste buds: Possible role in perception of intravascular gustatory stimuli. *Chem. Senses* **16:**69–80

Kashiwayanagi, M., Miyake, M., Kurihara, K. 1983. Voltage-dependent Ca^{2+} channel and Na^+ channel in frog taste cells. *Am. J. Physiol.* **244:**C82–C88

Kinnamon, S.C., Dionne, V.E., Beam, K.G. 1988. Apical localization of K^+ channels in taste cells provides the basis for sour taste transduction. *Proc. Natl. Acad. Sci. USA* **85:**7023–7027

Kinnamon, S.C., Roper, S.D. 1987. Passive and active membrane properties of mudpuppy taste receptor cells. *J. Physiol.* **383:**601–614

Kinnamon, S.C., Roper, S.D. 1988*a*. Evidence for a role of voltage-sensitive apical K^+ channels in sour and salt taste transduction. *Chem. Senses* **13:**115–121

Kinnamon, S.C., Roper, S.D. 1988*b*. Membrane properties of isolated mudpuppy taste cells. *J. Gen. Physiol.* **91:**351–371

Kinnamon, S.C., Roper, S.D., Beam, K.G. 1989. Calcium currents in isolated taste receptor cells of the mudpuppy. *Ann. N. Y. Acad. Sci.* **560:**112–115

Kolb, H.-A. 1990. Potassium channels in excitable and non-excitable cells. *Rev. Physiol. Biochem. Pharmacol.* **115:**51–89

L'Allemain, G., Paris, S., Pouyssegur, J. 1985. Role of a Na^+-dependent Cl$^-$/HCO_3^- exchange in regulation of intracellular pH in fibroblasts. *J. Biol. Chem.* **260:**4877–4883

Marty, A. 1989. The physiological role of calcium-dependent channels. *Trends Neurosci.* **12:**420–424

Ozeki, M. 1971. Conductance change associated with receptor potential of gustatory cell in rat. *J. Gen. Physiol.* **58:**688–699

Roper, S.D. 1983. Regenerative impulses in taste cells. *Science* **220:**1311–1312

Roper, S.D. 1989. The cell biology of vertebrate taste receptors. *Annu. Rev. Neurosci.* **12:**329–353

Roper, S.D., McBride, D.W. 1989. Distribution of ion channels on taste cells and its relationship to chemosensory transduction. *J. Membrane Biol.* **109:**29–39

Schiffman, S.S., McElroy, A.E., Erickson, R.P. 1980. The range of taste quality of sodium salts. *Physiol Behav.* **24:**217–224

Spielman, A.I., Mody, I., Brand, J.G., Whitney, G., MacDonald, J.F., Salter, M.W. 1989. A method for isolating and patch-clamping single mammalian taste receptor cells. *Brain Res.* **503:**326–329

Sugimoto, K., Teeter, J.H. 1990. Voltage-dependent ionic currents in taste receptor cells of the larval tiger salamander. *J. Gen. Physiol.* **96:**809–834

Yang, J., Roper, S. 1987. Dye-coupling in taste buds in the mudpuppy. *Necturus maculosus*. *J. Neurosci.* **7:**3561–3565

Received 6 March 1991; revised 21 May 1991