

Membrane Properties of Isolated Mudpuppy Taste Cells

SUE C. KINNAMON and STEPHEN D. ROPER

From the Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, Colorado 80523, and the Rocky Mountain Taste and Smell Center, University of Colorado Health Sciences Center, Denver, Colorado 80262

ABSTRACT The voltage-dependent currents of isolated *Necturus* lingual cells were studied using the whole-cell configuration of the patch-clamp technique. Nongustatory surface epithelial cells had only passive membrane properties. Small, spherical cells resembling basal cells responded to depolarizing voltage steps with predominantly outward K^+ currents. Taste receptor cells generated both outward and inward currents in response to depolarizing voltage steps. Outward K^+ currents activated at ~ 0 mV and increased almost linearly with increasing depolarization. The K^+ current did not inactivate and was partially Ca^{++} dependent. One inward current activated at -40 mV, reached a peak at -20 mV, and rapidly inactivated. This transient inward current was blocked by tetrodotoxin (TTX), which indicates that it is a Na^+ current. The other inward current activated at 0 mV, peaked at 30 mV, and slowly inactivated. This more sustained inward current had the kinetic and pharmacological properties of a slow Ca^{++} current. In addition, most taste cells had inwardly rectifying K^+ currents. Sour taste stimuli (weak acids) decreased outward K^+ currents and slightly reduced inward currents; bitter taste stimuli (quinine) reduced inward currents to a greater extent than outward currents. It is concluded that sour and bitter taste stimuli produce depolarizing receptor potentials, at least in part, by reducing the voltage-dependent K^+ conductance.

INTRODUCTION

Taste receptor cells are the peripheral transducing elements of gustatory sensation. Chemical stimuli presumably interact with microvillar processes on the apical region of the taste cell membrane, ultimately resulting in transmitter release and excitation of gustatory nerve fibers (Beidler, 1970; Cummings et al., 1987; Teeter and Brand, 1987). Although classic taste stimuli (sour, salty, sweet, and bitter chemicals) induce depolarizing receptor potentials in the taste cells of a variety of species (catfish: Teeter and Kare, 1974; frogs: Akaike et al., 1976; Okada et al., 1985; mudpuppies: West and Bernard, 1978; Kinnamon and

Address reprint requests to Dr. Sue C. Kinnamon, Dept. of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523.

Roper, 1986, 1988; rats: Ozeki, 1971; Sato and Beidler, 1983; mice: Tonosaki and Funakoshi, 1984), little is known about the mechanisms involved in the transduction process. This lack of information is due in part to the difficulty associated with maintaining stable intracellular impalements from the small taste cells of most species. Most previous investigators have reported that taste cells have low resting potentials, low input resistances, and electrically inexcitable membranes (for review, see Sato, 1980), which may, in part, be due to damage caused by microelectrode impalement.

Recent investigations (Roper, 1983; Kashiwayanagi et al., 1983; Avenet and Lindemann, 1986, 1987; Kinnamon and Roper, 1987*a*) using improved intracellular recording techniques have reported data that differ significantly from those of previous investigators. In particular, the taste cells of amphibians have been shown to have high resting potentials and input resistances, similar to those of neurons. More importantly, these taste cells generate action potentials with Na^+ and Ca^{++} components in response to depolarizing current injection. The role of voltage-dependent conductances and action potentials in taste transduction, however, remains unclear.

In this study, we have applied the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981) to isolated mudpuppy taste cells, so that the voltage-dependent currents underlying the action potential could be studied in isolation. This technique allows cells to be voltage- or current-clamped with a high degree of temporal resolution and minimal damage to the cells. We report here that mudpuppy taste receptor cells have several voltage-dependent ionic currents, including rapidly inactivating Na^+ currents, slowly inactivating Ca^{++} currents, and noninactivating K^+ currents. In addition, we present evidence that these currents are modulated in different ways by sour and bitter taste stimuli. Preliminary reports of some of these findings have been made (Kinnamon and Roper, 1987*b, c*).

METHODS

Preparation

Mudpuppies (*Necturus maculosus*) were obtained from commercial suppliers and housed at 10°C in large freshwater aquaria. They were fed goldfish and earthworms weekly.

Our procedure for obtaining isolated taste cells will be described elsewhere (Kinnamon, S. C., T. A. Cummings, and S. D. Roper, manuscript submitted for publication). Briefly, mudpuppies were rapidly decapitated and the lingual epithelium was stripped from the surface of the tongue using blunt dissection. The epithelium was stretched flat in a shallow chamber and incubated in amphibian physiological saline (APS) containing collagenase (1.5 mg/ml; type 3, CooperBiomedical, Inc., Malvern, PA) and albumin (1 mg/ml) for 35 min. After rinsing the tissue in APS, the epithelium was gently separated from the underlying connective tissue, leaving behind the more adhesive taste buds on prominent connective tissue papillae. Isolated taste buds were incubated in Ca-free APS for 10 min, or until taste cells began to separate. Isolated taste cells were drawn into fire-polished glass pipettes and plated onto glass coverslips that had been coated with concanavalin A (Maue and Dionne, 1987) or Cell-Tak (BioPolymers, Inc., Farmington,

CT). In some experiments, the stripped nongustatory surface epithelial cells were dissociated and plated onto coverslips for physiological recording.

Gigaseal Whole-Cell Recording Technique

Membrane current or voltage was recorded and analyzed using the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981). Electrodes were fabricated from microhematocrit capillary tubes (American Scientific Products, McGaw Park, IL) using a two-stage vertical patch electrode puller. It was not necessary to fire-polish the electrode tips to obtain seals, but the shanks were coated with Sylgard (Dow Corning Corp., Midland, MI) or dental periphery wax (Surgident, Ltd., Los Angeles, CA) to reduce electrode capacitance. The electrode resistance was typically 4–8 M Ω when the pipettes were filled with saline.

Taste cells were viewed at a magnification of 400 using an Olympus IMT2 inverted microscope. Recording electrodes were positioned with a hydraulic micromanipulator and seals ranging from 2 to 15 G Ω were obtained by applying negative pressure to the pipette. A whole-cell recording was obtained by applying further suction to the pipette; access to the cell interior was signaled by a large increase in capacitative current flowing in response to a voltage step applied to the pipette.

Whole-cell current or voltage was measured at room temperature using an Axopatch patch-clamping amplifier (Axon Instruments, Inc., Burlingame, CA). The signal was pre-filtered at 5 kHz and digitally recorded at 100- μ s intervals (unless otherwise indicated) with a computer system (11/73, Digital Equipment Corp., Marlboro, MA) equipped with a Cheshire data interface (Indec Systems, Inc., Sunnyvale, CA). Voltage pulses applied to the pipette were also generated by the computer. Membrane capacitance was estimated by integrating the capacitative transient and dividing by the amplitude of the voltage step; the membrane surface area was calculated assuming a specific membrane capacitance of 1 μ F/cm². The series resistance of the pipette was estimated by dividing the amplitude of the voltage step by the peak of the capacitative transient; values typically ranged from 10 to 20 M Ω . Both membrane capacitance and pipette series resistance were electronically canceled using the patch-clamp amplifier. Series resistance, however, could be compensated only 10–15%. Leak and linear capacitative currents flowing through the membrane were measured by a series of 25-mV hyperpolarizing voltage pulses applied to the pipette from the holding potential; these currents were subtracted from records by computer unless otherwise indicated.

Solutions and Taste Stimulation

Our standard APS consisted of 112 mM NaCl, 2 mM KCl, 8 mM CaCl₂, and 5 mM HEPES (buffered to pH 7.2 with NaOH), unless otherwise indicated; Ca⁺⁺ was elevated to improve the stability of the recordings. Drugs were dissolved in APS and bath-applied using a gravity perfusion system; exchange was complete in 5–15 s.

The intracellular pipette solution consisted of 80 mM K gluconate, 10 mM NaCl, 10 mM HEPES (buffered to pH 7.2 with KOH), and 10⁻⁷ M or 10⁻⁸ M free Ca⁺⁺ (buffered with 1 mM BAPTA), unless otherwise indicated.

For voltage-clamp experiments, taste stimuli were dissolved in APS and bath-applied. For current-clamp experiments, concentrated solutions of taste stimuli dissolved in APS were added to adjacent barrels of a multibarreled pipette (tip diameter, 1–2 μ m) placed \sim 20 μ m from the taste cells. The chemicals were pressure-ejected from the tip by a pulse of air applied to the back of the pipette with a picospritzer (General Valve Corp., Fair-

field, NJ). In these experiments, the concentration could be varied by changing the duration or pressure of the pulse applied to the back of the pipette. However, the absolute concentration of the stimulus at the receptor membrane was unknown.

RESULTS

Cell Types and Membrane Currents

Taste cells in the mudpuppy are found within taste buds on papillae spaced 1–2 mm apart in the lingual epithelium. Ultrastructural studies (Farbman and Yonkers, 1971; Delay and Roper, 1987) have shown that taste buds contain both light and dark taste cells, as well as basal cells. Basal cells in the mouse are thought to be precursors to taste receptor cells (Delay et al., 1986). Thus, it was

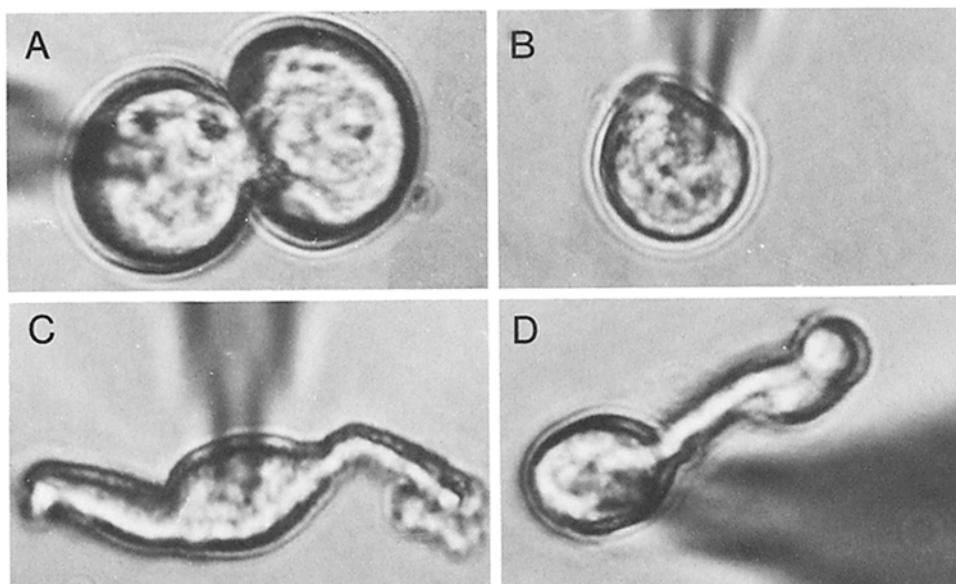


FIGURE 1. Photomicrographs of cells isolated from lingual epithelium. Epithelial cells (A), a basal cell (B), and taste cells (C and D) are illustrated. The shadow of the patch electrode can be seen in each micrograph. 1 cm = 10 μ m.

of interest to attempt to distinguish cell types and to compare membrane currents in the isolated taste cells. In addition, we have examined membrane currents in isolated nongustatory surface epithelial cells for comparison. Epithelial cells were obtained by dissociating the epithelial layer that was peeled from the underlying connective tissue; care was taken to use tissue that did not contain taste buds.

Dissociated surface epithelial cells are illustrated in Fig. 1 A and isolated cells from taste buds are illustrated in Fig. 1, B–D. Although surface epithelial cells were always spherical, cells from taste buds had a wide range of morphologies ranging from spherical cells (Fig. 1 B) to spindle-shaped cells with elongate pro-

cesses (Fig. 1, *C* and *D*). Small spherical cells appeared to form a distinct sub-population with surface areas $<3,500 \mu\text{m}^2$. Larger cells (surface area $>4,500 \mu\text{m}^2$) tended to be spindle-shaped or oblong. Because these small spherical cells resembled the size and shape of basal cells in situ (Farbman and Yonkers, 1971; Delay, R. J., and S. D. Roper, manuscript in preparation) and because the membrane currents of these smaller cells differed significantly from membrane currents of larger cells (see below), we have tentatively identified the small spherical isolated cells as "basal cells." The larger, spindle-shaped or oblong isolated cells were classified as "taste cells." We could not distinguish dark and light taste cells.

All cell types from dissociated lingual epithelium readily formed seals with patch pipettes. The passive membrane properties of the various cell types are summarized in Table I and Fig. 2. The voltage-activated membrane currents of the different cell types varied in a characteristic manner. The least complex currents were recorded from surface epithelial cells, which displayed only passive membrane properties (data not shown). Typical voltage-clamp recordings from

TABLE I
Resting Membrane Properties of Isolated Lingual Cells

Cell type	Surface area μm^2	Resting potential mV	Input resistance $M\Omega$
Epithelial	$3,574 \pm 1,047$	-19.6 ± 4.9	1.7 ± 0.9
Basal	$2,296 \pm 766$	-34.4 ± 10.5	3.7 ± 2.5
Taste	$5,390 \pm 1,528$	-65.0 ± 27	1.4 ± 0.9

All values are expressed as the means \pm 1 SD. Sample numbers were 8 (epithelial), 10 (basal), and 33 (taste). The resting potential was the zero-current potential and input resistances were measured at a holding potential of -100 mV . All values were different from each other ($p < 0.01$), except the input resistance of epithelial vs. basal cells ($p < 0.05$) and the input resistance of epithelial vs. taste cells (NS).

basal cells are illustrated in Fig. 3. In response to a superimposed sequence of depolarizing voltage steps from a holding potential of -100 mV , basal cells exhibited predominantly outward currents. These currents were blocked by bath perfusion with 8 mM tetraethylammonium bromide (TEA), which indicates that they are K^+ currents. Outward currents in basal cells activated slowly; the half-activation time for a pulse to $+60 \text{ mV}$ was 5 ms (Fig. 3A). In addition to K^+ currents, some basal cells exhibited small inward currents that always had a slowly inactivating component and occasionally a rapidly inactivating component as well. The more sustained inward current was reversibly blocked with 1 mM CdCl_2 , which indicates that it is a Ca^{++} current. The transient inward current was reversibly blocked by 100 nM tetrodotoxin (TTX), which indicates that it is an Na^+ current. Of the basal cells examined that had inward currents (5 out of 10), all had Ca^{++} currents, but only 2 of these cells also had Na^+ currents. The total peak inward current was never more than 60% of the total K^+ current at $+40 \text{ mV}$. The current-voltage relationship for a basal cell with a Ca^{++} current is shown in Fig. 3B. Outward currents activated at $+10 \text{ mV}$ and continued to

increase almost linearly with increasing depolarization; the Ca^{++} current activated at -10 mV, peaked at $+20$ mV, and declined with further depolarization.

Typical recordings from taste cells are illustrated in Fig. 4. In response to a sequence of depolarizing voltage steps from a holding potential of -100 mV, taste cells usually exhibited large inward and outward currents. In contrast to basal cells, the total peak inward current of taste cells was two to three times the total K^+ current at $+40$ mV. Perfusion with 8 mM TEA blocked outward

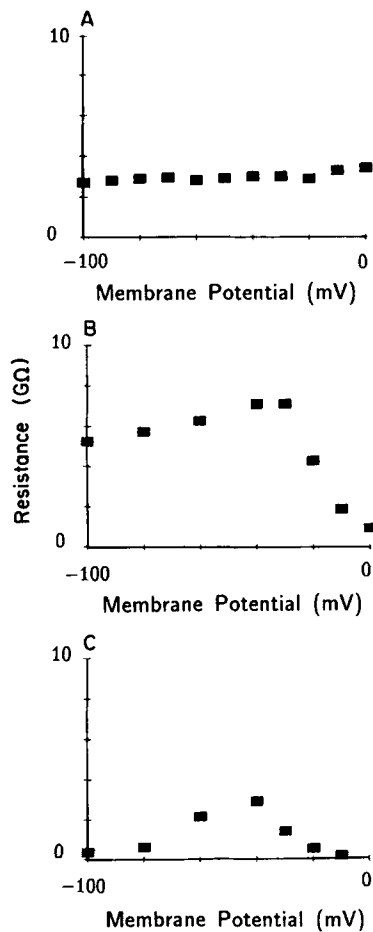


FIGURE 2. Relationship between membrane resistance and membrane potential for an epithelial cell (A), a basal cell (B), and a taste cell (C). Resistances were calculated from 25-mV hyperpolarizing voltage pulses (17.5 ms duration) applied to the pipette at each holding membrane potential.

K^+ currents and revealed two types of inward currents (Fig. 4 B): a transient, TTX-sensitive Na^+ current, and a slowly inactivating, CdCl_2 -sensitive Ca^{++} current. Although all taste cells had large Na^+ currents, the magnitude of the Ca^{++} current and the outward K^+ current varied greatly from cell to cell. Maximal Ca^{++} currents ranged from 10 to 50% of the maximal Na^+ current, and outward K^+ currents at $+40$ mV were 20–150% of the maximal Na^+ current. The magnitudes of Ca^{++} and K^+ currents were not correlated in the different taste

cells; i.e., cells with large Ca^{++} currents did not necessarily have large outward currents.

Taste cells were electrically excitable and generated action potentials in response to injections of small pulses of depolarizing current (Fig. 4 C). The duration of the action potential was variable in different taste cells. Taste cells with large Ca^{++} currents usually had long-duration action potentials, but we rarely observed the large Ca^{++} -dependent afterpotentials that have been recorded from taste cells in intact lingual epithelium (Kinnamon and Roper, 1987b). The voltage-dependent currents of the taste cell membrane are described in more detail below.

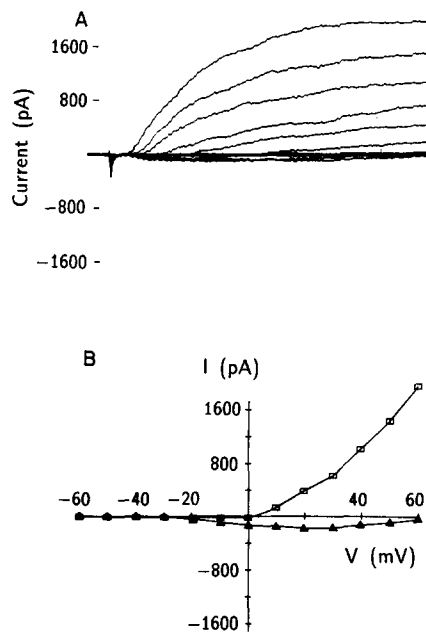


FIGURE 3. Whole-cell currents from a basal cell. (A) Currents were recorded in response to a sequence of depolarizing voltage pulses (17.5 ms duration) applied to the pipette from a holding potential of -100 mV. Voltage pulses were in 10-mV steps from -60 to $+60$ mV. Outward current is positive. Leak and linear capacitive currents were subtracted in this and subsequent figures unless otherwise indicated. (B) The peak current-voltage relationship is shown for the cell in A. The open squares are the peak outward currents at each pulse potential. The filled triangles are the peak Ca^{++} currents at each pulse potential, obtained after bathing the cell in 8 mM TEA.

Na⁺ Currents

The large cell size and uncompensated series resistance of the pipette prevented a detailed analysis of Na^{+} currents in taste cells. Nevertheless, Na^{+} currents were present in most taste cells, and a typical recording is illustrated in Fig. 5. Na^{+} currents were isolated by bathing the preparation in 8 mM TEA to block outward K^{+} currents and replacing Ca^{++} in the bath with Mg^{++} , or by selecting cells with negligible Ca^{++} currents (cf. Fig. 6). The membrane was held at -100 mV and a sequence of depolarizing voltage steps was applied to the pipette. A pulse to -20 mV, for example, elicited an inward current that reached a peak in ~ 2 ms and was almost completely inactivated in 5 ms (Fig.

5 A). Inactivation was voltage dependent, with larger depolarizations resulting in faster inactivation. The peak current-voltage relationship is illustrated in Fig. 5 B. Currents usually activated at -40 mV, reached a peak between -20 and 0 mV, and slowly declined with further depolarization. Normalized peak Na^+ currents are plotted against the holding potential in Fig. 5 C. Currents were half-

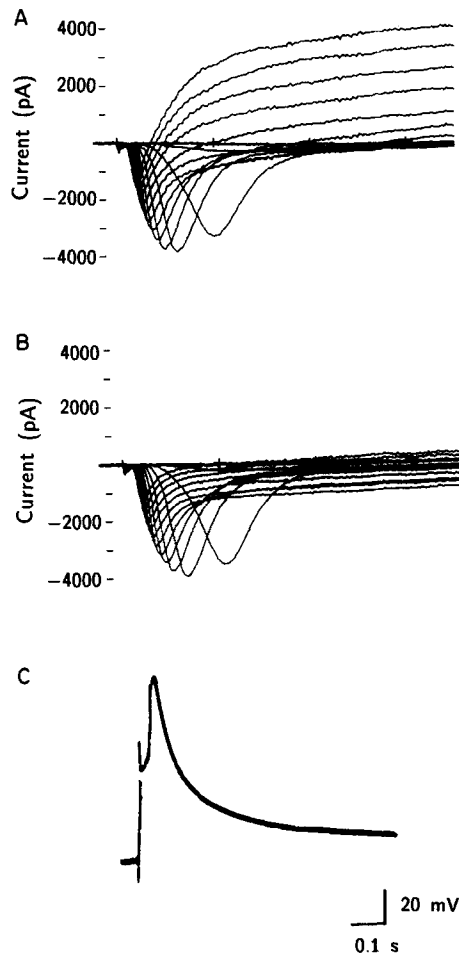


FIGURE 4. Whole-cell voltage-clamp and current-clamp recordings from taste cells. (A) Voltage-clamp recordings in normal ACSF. (B) Recordings from the same taste cell after bath perfusion with 8 mM TEA. The holding potential was -100 mV and the membrane was stepped in 10-mV steps from -60 to $+80$ mV (pulse duration was 17.5 ms). Note that TEA blocked the outward current and revealed the presence of a sustained inward current. (C) An action potential was elicited from a different isolated taste cell in response to a brief pulse of current injection.

maximal at a holding potential of approximately -65 mV and completely inactivated at holding potentials positive to -20 mV. Thus, only approximately half of the Na^+ current is available at the resting potential. Although the magnitude of the peak Na^+ current varied from cell to cell, there was little variation in the shape of the current-voltage relationship or in the steady state inactivation of Na^+ currents.

The Na^+ current was reversibly blocked by 100–200 nM TTX or by replacement of Na^+ with choline. The current was unaffected, however, by 1 mM CdCl_2 or by replacement of Ca^{++} with Mg^{++} . Thus, there is no Ca^{++} component to the transient inward current of the taste cell membrane. In addition, we examined the effect of 100 μM amiloride on Na^+ currents, since amiloride-sensitive

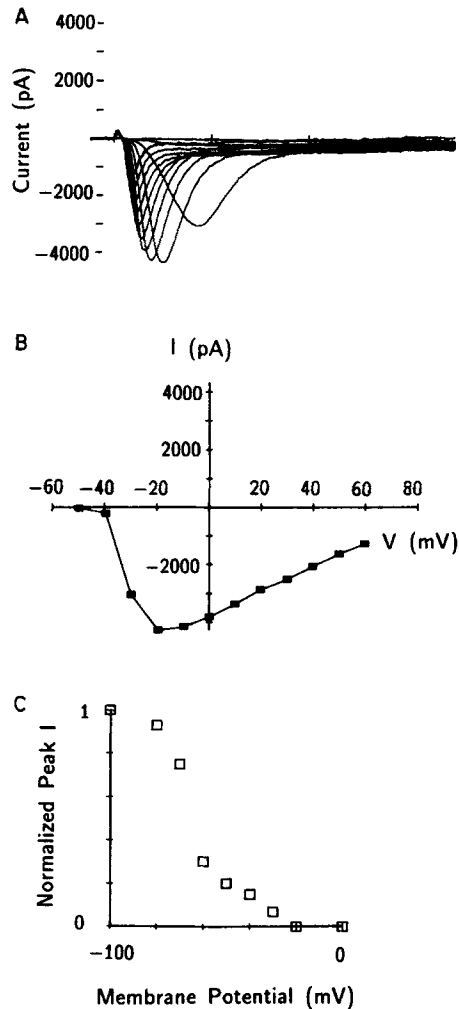


FIGURE 5. Na^+ currents in taste cells. (A) Currents were recorded from a taste cell bathed in 8 mM TEA to block outward K^+ currents. The holding potential was -100 mV, and the membrane was stepped from -50 to $+60$ mV in 10-mV steps (pulse duration was 17.5 ms). In addition to the Na^+ current, this cell had only small sustained inward Ca^{++} currents. (B) Peak inward currents from the same taste cell are plotted against pulse potential. (C) The steady state inactivation is shown for a different taste cell. The peak currents at each holding membrane potential were normalized to the peak current at a holding potential of -100 mV and plotted against holding membrane potential.

channels have been implicated in NaCl taste transduction (Heck et al., 1984). There was no effect of amiloride on the voltage-dependent Na^+ currents.

Ca⁺⁺ Currents

Typical Ca^{++} currents from a taste cell are illustrated in Fig. 6. Ca^{++} currents were isolated by bathing the cells in 8 mM TEA to block outward K^+ currents

and 1 μM TTX to block Na^+ currents. A sequence of depolarizing voltage pulses from a holding potential of -100 mV elicited the Ca^{++} current. The current activated 1–2 ms after the onset of the voltage pulse, reached a peak at 5–10 ms, and slowly inactivated; inactivation was still incomplete at 60 ms after the onset of the voltage pulse (Fig. 6 A). Inactivation was not Ca^{++} dependent, since currents also inactivated with Ba^{++} as the charge carrier (Fig. 6 C) and when the

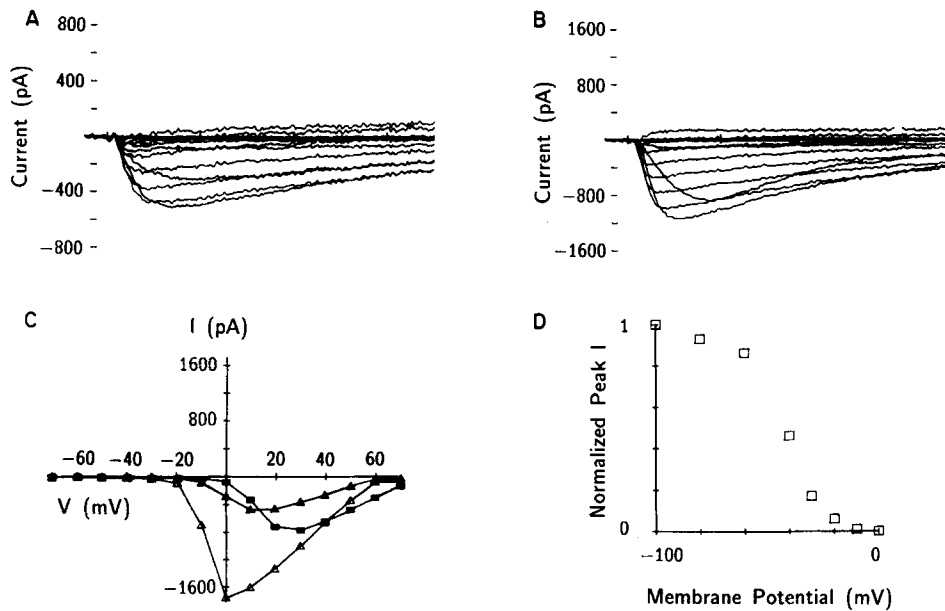


FIGURE 6. Ca^{++} currents in taste cells. Ca^{++} currents were isolated by bathing taste cells in 100 nM TTX to block Na^+ currents and in 8 mM TEA to block outward K^+ currents. The holding potential was -100 mV and the membrane was stepped in 10-mV steps from -60 to $+80$ mV (pulse duration was 70 ms). (A) Currents were recorded in 8 mM Ca^{++} . (B) Peak current-voltage relationship for Ca^{++} currents in a different taste cell. The filled triangles are the peak Ca^{++} currents when the bath contained 2 mM Ca^{++} . The squares are the peak Ca^{++} currents when the bath contained 8 mM Ca^{++} . The open triangles are the peak Ba^{++} currents when 8 mM Ba^{++} was substituted for Ca^{++} . All records are from the same cell. (C) Currents were recorded in 8 mM Ba^{++} (Ba^{++} replaced Ca^{++} in the bath). (D) Steady state inactivation of Ba^{++} currents in a different taste cell. Peak currents at each holding potential are normalized to the peak current at a holding potential of -100 mV. The bath contained 8 mM Ba^{++} .

intracellular pipette solution contained 25 mM EGTA and no added Ca^{++} . Fig. 6 B illustrates the current-voltage relationship for the Ca^{++} current with 2 mM Ca^{++} (filled triangles), 8 mM Ca^{++} (filled squares), and 8 mM Ba^{++} (open triangles) as charge carriers. In standard APS (8 mM Ca^{++}), the current activated at 0 mV, reached a peak at $+30$ mV, and slowly decreased with further depo-

larization. The peak Ca^{++} current was reduced by $\sim 40\%$ in 2 mM Ca^{++} . With 8 mM Ba^{++} substituted for Ca^{++} , the current was about twice that in Ca^{++} and the current-voltage relationship was shifted to the left. Normalized peak currents with 8 mM Ba^{++} as the charge carrier are plotted against holding potential in Fig. 6 D. The current was half-maximal at a holding potential of -40 mV and completely inactivated at a holding potential of -10 mV. Thus, $\sim 90\%$ of the current can be activated at the normal resting potential. Similar results were obtained with 8 mM Ca^{++} .

Ca^{++} currents were reversibly blocked by 0.1 mM CdCl_2 , 8 mM MnCl_2 , and by substitution of Mg^{++} for Ca^{++} in the bath. The dihydropyridine nifedipine had no effect on Ca^{++} currents at a concentration of 1 μM , but 10 μM nifedipine reversibly reduced peak Ca^{++} currents by $\sim 20\%$. The magnitude of the Ca^{++} current varied greatly in different taste cells, ranging from ~ 200 to $>1,600$ pA. The kinetics, pharmacology, and current-voltage relationship of Ca^{++} currents, however, were similar in the different taste cells.

Outward Currents

Voltage-dependent outward currents were isolated by bathing the cells in Ca^{++} -free, 8 mM Mg^{++} APS to block Ca^{++} currents and holding the membrane at -40 mV to inactivate Na^+ currents. A typical recording is illustrated in Fig. 7 A. Outward currents activated in 2–5 ms (the half-activation time for the pulse to $+60$ mV was 2.5 ms), reached a peak in 10–20 ms, and did not inactivate. Pulse potentials lasting 3–10 s showed no decrement in amplitude (Fig. 7 B). The current-voltage relationship is shown in Fig. 7 C; current activated at 0 mV and increased almost linearly with depolarization. Since this outward current is noninactivating, it probably accounts for the decrease in membrane resistance observed at depolarized holding potentials (Fig. 2 C). However, the decrease in resistance occurred at holding potentials positive to -40 mV, and outward currents recorded in response to depolarizing voltage steps usually activated at 0 mV. It is possible that outward currents activate very slowly at potentials negative to 0 mV, requiring several seconds for full activation.

Perfusion with 8 mM TEA, 8 mM Ba^{++} , or replacement of K^+ and Cs^+ in the pipette blocked 90–95% of the outward current when pulse potentials did not exceed $+80$ mV, which indicates that it is a K^+ current. Outward currents were unaffected by 10 mM 3,4-diaminopyridine.

One type of noninactivating, voltage-dependent K^+ current is the Ca^{++} -dependent K^+ current found in a variety of excitable cells (for review, see Meech, 1978). To determine whether a Ca^{++} -dependent K^+ current was present in taste cells, we examined the effect of replacing Ca^{++} with Mg^{++} on the magnitude of the outward current. In some experiments, Ca^{++} removal partially reduced the outward current. The magnitude of the effect was not correlated with the magnitude of the Ca^{++} current; some cells with large Ca^{++} currents had outward currents unaffected by Ca^{++} removal. Since isolated taste cells rarely produced the Ca^{++} -dependent afterpotentials observed in taste cells in intact lingual epithelium (Kinnamon and Roper, 1987a), we hypothesized that pipette BAPTA (1 mM) was buffering Ca^{++} influx so that Ca^{++} -dependent cur-

rents were not activated. To test this hypothesis, we recorded outward currents using a pipette solution containing 0.1 mM BAPTA and no added Ca^{++} . We could hold cells for only a few minutes under these conditions, since the cells became leaky and the pipette series resistance increased greatly. In many cells, however, the current-voltage relationship at the onset of the recording showed

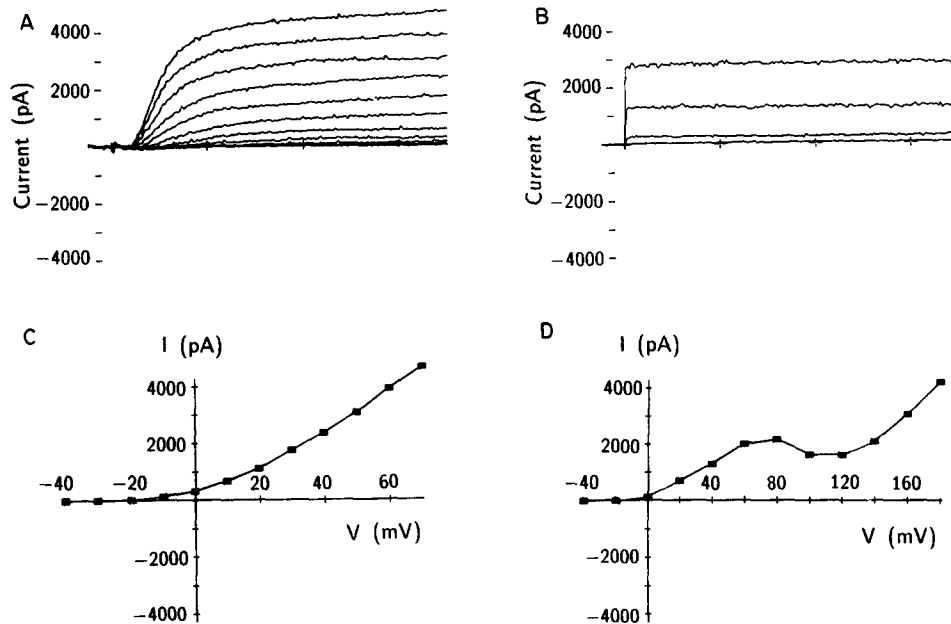


FIGURE 7. Outward K^+ currents in taste cells. Outward currents were isolated by bathing cells in Mg^{++} APS (Mg^{++} substituted for Ca^{++}) to block Ca^{++} currents. (A) The holding potential was -43 mV (to inactivate Na^+ currents) and the membrane was stepped in 10-mV steps from -40 to $+70$ mV (pulse duration was 17.5 ms). (B) Outward currents in response to long-duration (3-s) voltage pulses are shown for a different taste cell. The holding potential was -99 mV and the membrane was stepped in 30-mV steps from -20 to $+70$ mV. The transient Na^+ current cannot be seen because of the slow sampling rate used in this experiment. Leak currents were not subtracted. (C) Peak current-voltage relationship for outward currents from the taste cell in A. The pipette contained 10^{-8} M Ca^{++} , buffered with 1 mM BAPTA. (D) Steady state current-voltage relationship for a different taste cell; currents were measured at the end of the voltage pulse (17.5 ms). The pipette contained 0.1 mM BAPTA and no added Ca^{++} . The holding potential was -101 mV and the membrane was stepped from -40 to $+180$ mV in 20-mV steps. The bath contained 8 mM Ca^{++} .

a region of negative slope conductance near or slightly above the Ca^{++} equilibrium potential, with current increasing linearly at more positive potentials (Fig. 7D). This type of current-voltage relationship has been observed in a variety of vertebrate neurons and is thought to result from co-activation of a Ca^{++} -dependent K^+ conductance and a delayed rectifier K^+ conductance (Adams et al.,

1982; Galvan and Sedlmeir, 1984). We were not able to hold cells for a sufficient period to change solutions, so the Ca^{++} -dependent basis of the N-shaped current-voltage relationship could not be determined.

Currents through Inwardly Rectifying K^+ Channels

Because the membrane resistance of taste cells often decreased with hyperpolarization from the resting potential (Fig. 2 C), we suspected the presence of inwardly rectifying K^+ channels. Cells were bathed in elevated K^+ (10 mM) to enhance inward K^+ current flow, 1 mM CdCl_2 to block Ca^{++} currents, and 100 nM TTX to block Na^+ currents. A sequence of hyperpolarizing and depolarizing voltage steps from the K^+ reversal potential (-50 mV) elicited the currents shown in Fig. 8 A. Currents were larger in response to hyperpolarizing voltage

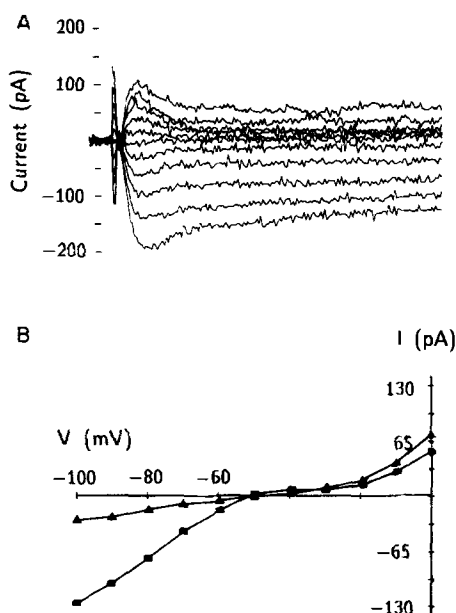


FIGURE 8. Currents through inwardly rectifying K^+ channels in taste cells. Taste cells were bathed in 10 mM KCl , 100 nM TTX, and 1 mM CdCl_2 to block current through Na^+ and Ca^{++} channels, and to enhance current through the inward rectifier. (A) The holding potential was -48 mV and the membrane was stepped from -100 to 0 mV in 10-mV steps (pulse duration was 17.5 ms). Leak currents were not subtracted. (B) Steady state current-voltage relationship for the same taste cell; currents were measured at the end of the voltage pulse. The squares represent current in 10 mM K^+ ; the triangles represent current when Rb^+ was substituted for K^+ .

steps than to depolarizing voltage steps. This is more clearly shown in the current-voltage relationship (squares, Fig. 8 B); the inward rectification was completely blocked by replacement of K^+ with Rb^+ (triangles, Fig. 8 B), an ion that does not pass through the inward rectifier (Hagiwara and Takahashi, 1974). Inward rectification was also blocked by 8 mM Ba^{++} , but was unaffected by 8 mM TEA. The amount of inward rectification varied greatly among different taste cells, with some cells having nearly linear current-voltage relationships under these conditions and other cells showing pronounced inward rectification.

Response to Sour Taste Stimulation

Both weak acids (which taste sour to humans) and quinine (which tastes bitter to humans) applied to the tongue elicit a strong response of the glossopharynx-

geal nerve in the intact mudpuppy (McPheeters and Roper, 1985), so these chemical stimuli were used on the isolated taste cells. We focused on the chemosensory responses of the taste cells in this study and did not examine the

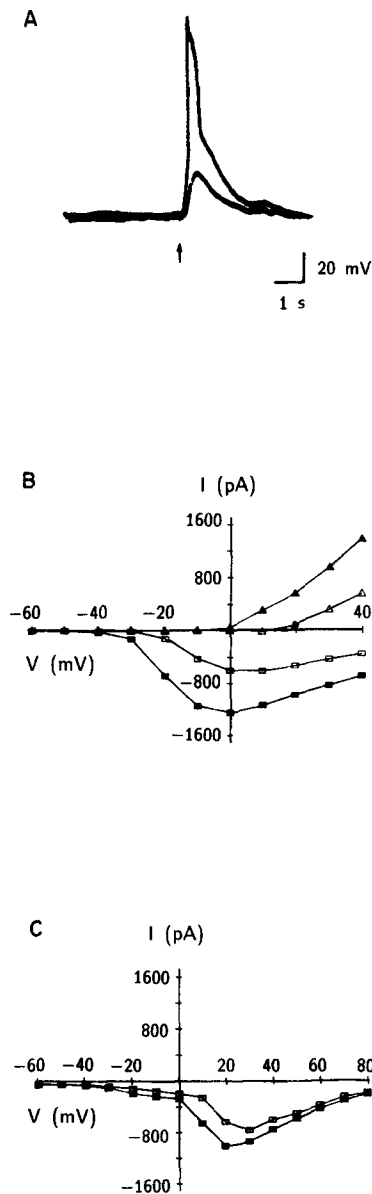


FIGURE 9. Responses in isolated taste cells evoked by sour taste stimulation. (A) Voltage response to citric acid. At the arrow, citric acid was focally applied to the taste cell membrane from a puffer pipette placed $\sim 20 \mu\text{m}$ from the taste cell; the pipette contained 1 mM citric acid. Two different concentrations, achieved by altering the duration of the air pressure pulse (not shown) elicited the two responses shown here. (B) Peak current-voltage relationship obtained from voltage-clamp recordings from a different taste cell, bathed in 1 mM CdCl_2 to block Ca^{++} currents. The holding potential was -100 mV and voltage steps from -60 to $+40 \text{ mV}$ were applied to the pipette (pulse duration was 17.5 ms). The filled squares represent the peak inward (Na^+) current and the filled triangles represent the peak outward (K^+) current in control APS. The open squares and open triangles represent peak inward and outward currents, respectively, during bath perfusion with 0.01 mM citric acid (pH 4.8). (C) Current-voltage relationship obtained from voltage-clamp recordings from the same taste cell, bathed in 8 mM TEA to block outward currents and in 100 nM TTX to block Na^+ currents. The filled squares are peak Ca^{++} currents in control APS; the open squares are peak Ca^{++} currents during bath perfusion with 0.01 mM citric acid.

responses of basal cells or surface epithelial cells. Taste cells at the zero-current (resting) potential responded to focally applied citric acid (1 mM in pipette) with depolarizing receptor potentials or long-duration action potentials (Fig. 9A). Only a few cells responded with action potentials, even when resting potentials

were large, and voltage-clamp recordings revealed the presence of large Na^+ currents in the absence of taste stimulation. Responses were graded; larger depolarizations resulted from higher concentrations of stimulus owing to longer-duration stimulus applications. Although all taste cells showed some response to citric acid, the magnitude of the response was variable.

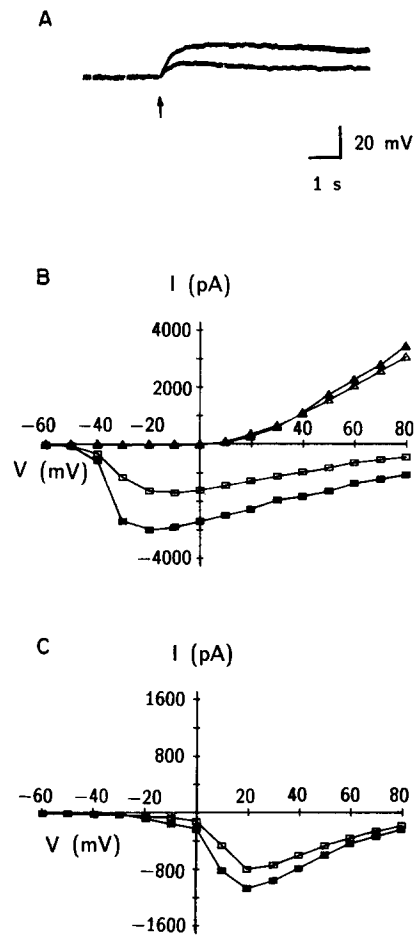


FIGURE 10. Responses in isolated taste cells evoked by bitter taste stimulation. (A) Voltage recording from a taste cell in response to focal stimulation with quinine hydrochloride, applied at the arrow. The puffer pipette contained 10 mM quinine; the two traces represent responses to two different concentrations of quinine, achieved as above (Fig. 9 A). (B) Peak current-voltage relationship obtained from voltage-clamp recordings from a different taste cell bathed in Mg^{++} APS to block Ca^{++} currents. The filled squares represent peak inward and filled triangles represent peak outward currents in control APS. The open squares and triangles represent peak inward and outward currents, respectively, during bath perfusion with 0.1 mM quinine. The holding potential was -100 mV and 10-mV voltage steps from -60 to $+80$ mV were applied to the pipette (pulse duration was 17.5 ms). (C) Current-voltage relationship obtained from voltage-clamp recordings from a different taste cell, bathed in TEA and TTX to isolate Ca^{++} currents. The filled squares represent peak Ca^{++} currents in normal APS; the open squares represent Ca^{++} currents during bath perfusion with 0.1 mM quinine.

The effects of bath-applied citric acid (0.01 mM, pH 4.8) on voltage-activated currents were recorded and used to produce the current-voltage curves illustrated in Fig. 9, B and C. The results from a typical cell, bathed in 1 mM CdCl_2 to block Ca^{++} currents, are illustrated in Fig. 9 B; Na^+ and K^+ currents were resolved by temporal displacement. Citric acid reduced peak outward K^+ currents by $\sim 60\%$ and peak Na^+ currents by $\sim 50\%$ without shifting the voltage

dependence. The current-voltage relation for Ca^{++} currents, isolated by bathing cells in 8 mM TEA and 100 nM TTX, is shown in Fig. 9 C. Peak Ca^{++} currents were reduced by ~40%, with little or no effect on their voltage dependence. There was no effect of citric acid (pH 4.8) on currents through the inwardly rectifying K^+ channel, but higher concentrations (0.1 mM, pH 3.2) did reduce this conductance (data not shown).

To determine whether the reduction in outward current contributed to the receptor potential, isolated taste cells were bathed in 8 mM TEA and focally stimulated with citric acid. TEA depolarized the resting membrane potential by ~20 mV and reduced, but did not always completely block, the receptor potential. These data suggest that citric acid produces depolarizing receptor potentials, at least in part, by blocking voltage-dependent K^+ channels that are partially activated at the resting membrane potential (Kinnamon and Roper, 1986, 1988). The reduction of inward currents by citric acid may explain why isolated taste cells rarely generated action potentials in response to citric acid taste stimulation.

Response to Bitter Taste Stimulation

Most taste cells responded to focally applied quinine hydrochloride (10 mM in pipette) with a graded, long-duration depolarization (Fig. 10 A). There was great variability from cell to cell in response to quinine; some cells failed to respond and other cells responded with depolarizations of several millivolts.

The effects of bath-applied quinine (0.1 mM) on voltage-activated currents were recorded and used to produce the current-voltage relations shown in Fig. 10, B and C. Quinine reduced the peak outward current by ~15% (Fig. 10 B). In contrast, peak Na^+ currents were reduced by ~50% (Fig. 10 B) and peak Ca^{++} currents were reduced by ~30% (Fig. 10 C) by quinine. Thus, quinine reduced inward currents to a greater extent than outward currents. Larger concentrations of quinine (1–10 mM) blocked outward as well as inward currents. There was no effect of 0.1 mM quinine on the inward-rectifier K^+ currents, but larger concentrations were not tested.

To determine whether the reduction in outward current contributed to the transduction event, cells were bathed in 8 mM TEA to block outward currents and focally stimulated with quinine. The receptor potential was only partially blocked by the TEA. These data suggest that quinine produces depolarizing receptor potentials in part by blocking the voltage-activated K^+ conductance; however, an additional, non-voltage-dependent conductance may be modulated as well.

DISCUSSION

The data presented here confirm our previous observations obtained from intracellular recordings of *Necturus* taste cells in intact lingual epithelia (Roper, 1983; Kinnamon and Roper, 1986, 1987a, 1988) and extend those findings to isolated taste cells. Isolated taste cells have substantial resting potentials and input resistances, generate action potentials with Na^+ and Ca^{++} components, and have a voltage-sensitive K^+ conductance (Roper, 1983; Kinnamon and Roper, 1986, 1987a, 1988). In this study, we have characterized the voltage-dependent currents of the taste cell membrane and have provided evidence for the involve-

ment of a voltage-dependent K^+ current in the transduction of sour and bitter taste stimuli.

Na^+ currents in taste cells are similar to the Na^+ currents first described by Hodgkin and Huxley (1952), and since then in many excitable membranes (for review, see Hille, 1984); they are blocked by nanomolar concentrations of TTX and show the expected voltage-dependent kinetics of activation and inactivation. These properties suggest that the Na^+ current is responsible for the upstroke of the action potential in taste cells, just as it is in other excitable tissues. The half-inactivation voltage of -65 mV (the typical resting potential of taste cells *in situ* and *in vitro*) may explain the observation that taste cells usually generate only a single action potential to a maintained depolarizing stimulus (Kinnamon and Roper, 1987*a*). In addition, it may explain why previous investigators failed to observe action potentials in the small taste cells of mammals (Ozeki, 1971; Sato and Beidler, 1983; Tonosaki and Funakoshi, 1984). The resting potentials of taste cells in those studies were approximately -40 mV, probably owing to damage from intracellular impalement. At this resting potential, our studies show that almost all of the Na^+ conductance is inactivated in the mudpuppy.

Ca^{++} currents in taste cells resemble the slow Ca^{++} currents described in a variety of excitable cells (Nowycky et al., 1985; Bean, 1985; Sturek and Hermsmeyer, 1986) in that they inactivate slowly, require strong depolarization for activation, are larger in Ba^{++} than in Ca^{++} , and are blocked by low concentrations of Cd^{++} . They differ from other slow Ca^{++} currents in that they are relatively insensitive to dihydropyridines and exhibit voltage-dependent, rather than Ca^{++} -dependent, inactivation. The relatively strong depolarization required for activation and the slow inactivation suggest that these Ca^{++} currents contribute to the plateau of the action potential (Roper, 1983; Kinnamon and Roper, 1987*a*). Transmitter release from chemically stimulated taste cells could be caused by a rise in intracellular Ca^{++} brought on by large depolarizations or action potentials.

At least two types of K^+ currents were observed in taste cells. One type activates with depolarization and resembles the delayed rectifier K^+ current (for review, see Hille, 1984). It is tempting to speculate that this voltage-dependent, noninactivating K^+ current seen in isolated cells is the same "resting" K^+ current that we previously observed in intact lingual epithelium. In the intact tissue, this K^+ current is blocked by TEA or by hyperpolarization from the resting potential (Kinnamon and Roper, 1986, 1987*a*, 1988). If the two K^+ conductances are the same, the voltage dependence *in vitro* differs slightly from that *in situ*. Larger depolarizations are required for activation of the current in the isolated taste cells (0 mV, cf. Fig. 7*C*) than in cells of intact lingual epithelium (at or slightly below resting potential). It is likely that at least a portion of this K^+ current is Ca^{++} dependent. Since we observed Ca^{++} -dependent afterpotentials in the taste cells from intact lingual epithelium but rarely in isolated taste cells, it is likely that the Ca^{++} -dependent K^+ currents were not often activated *in vitro*. The absence of the Ca^{++} -dependent K^+ current *in vitro* could account for the stronger depolarizations required for activation of the K^+ current in isolated taste cells, relative to our observations *in situ*. However, TEA depolarized the resting potential, even in isolated taste cells. Thus, at least a small portion of this underlying K^+ conductance must be activated at rest. In addition, this con-

ductance is clearly involved in action potential repolarization, since TEA prolongs the duration of action potentials in taste cells (Kinnamon and Roper, 1987*a*).

The other K^+ current that was observed in the isolated taste cells is activated by hyperpolarization and resembles the inward-rectifier K^+ current first reported in skeletal muscle by Katz (1949) and more recently studied in starfish eggs (Hagiwara and Takahashi, 1974) and spinal cord neurons (Stanfield et al., 1985). Since the underlying conductance is activated by hyperpolarization and is noninactivating, it clearly is partially activated at the resting potential. We doubt that it is involved in chemosensory transduction because in previous studies we found that the depolarization produced by KCl or citric acid applied to the apical membrane was blocked by hyperpolarization (Kinnamon and Roper, 1986, 1988). These data suggest that inward rectifier K^+ channels are restricted to the basolateral membrane, where they probably contribute to stabilizing the resting potential.

In the only other voltage-clamp study on taste cells, Avenet and Lindemann (1986, 1987) found that isolated frog taste cells possessed voltage-sensitive, TTX-sensitive Na^+ currents and voltage-activated outward K^+ currents with properties similar to those of mudpuppy taste cells. In addition, quinine reduced Na^+ and K^+ currents in a manner analogous to that of mudpuppy taste cells. In their studies, however, no evidence of Ca^{++} currents was found, even though frog taste cells generate action potentials with a Ca^{++} component (Kashiwayanagi et al., 1983).

There was a great deal of variability in the types and magnitudes of membrane currents found in the different cells isolated from taste buds. It is tempting to speculate that this variability represents different stages of development of taste receptor cells. In the mouse, taste receptor cells are thought to develop continually from basal cells; dark cells develop first and later mature into light cells (Delay et al., 1986). Complete turnover is thought to take 10 d to 2 wk. Although the developmental sequence is not yet known in the mudpuppy, the same cell types exist (Farbman and Yonkers, 1971; Delay and Roper, 1987). We found that basal cells had predominantly outward currents; if inward currents were present, they were usually Ca^{++} currents. If basal cells in the mudpuppy are indeed the precursor to taste cells, these data suggest that K^+ currents develop first, followed by Ca^{++} currents, and finally by Na^+ currents. Developing amphibian neurons show similar changes; action potentials shift from primarily Ca^{++} dependent to primarily Na^+ dependent (reviewed in Spitzer, 1979, 1985). During this time, the delayed rectifier K^+ currents (Barish, 1986) and Ca^{++} -activated K^+ currents (Blair and Dionne, 1985) increase in magnitude and activate more rapidly. We observed similar differences in the kinetics of the outward current in basal cells and taste cells (compare Figs. 3*A* and 7*A*). We also observed large variations in the magnitude of Ca^{++} and K^+ currents in the different taste cells. It will be of interest to determine whether these differences can be correlated to differences in taste cell type.

What are the roles of voltage-dependent conductances in taste transduction? Our data suggest that sour and bitter taste stimuli are transduced into receptor potentials at least in part by blocking or reducing a resting, voltage-dependent

K⁺ conductance. Although both types of taste stimuli produced depolarizing receptor potentials, the kinetics of the responses to the two stimuli were different. In particular, the response to bitter stimuli had a slower time course and was more prolonged relative to the response to sour stimuli. One caveat with the experiments on isolated cells is that taste stimuli were applied to the entire membrane, rather than just the apical region. Although we have shown in previous experiments that the apical membrane contains a K⁺ conductance that is blocked by hyperpolarization and TEA (Kinnamon and Roper, 1986, 1988), we do not know how Na⁺ and Ca⁺⁺ currents are segregated between apical and basolateral membranes. It will therefore be important to determine whether these kinetic differences are maintained when taste stimuli are restricted to the apical membrane.

Intracellular recordings have been made from the taste cells of other species in response to sour and bitter stimuli. Rat (Ozeki, 1971), mouse (Tonosaki and Funakoshi, 1984), and frog (Akaike et al., 1976) taste cells responded to bitter (quinine) stimulation with membrane depolarizations accompanied by a decrease in membrane conductance, and to sour (weak acids) stimulation with depolarizations accompanied either by small increases in membrane conductance or by little change in conductance. On the basis of our voltage-clamp recordings, we would predict decreases in membrane conductance to both taste stimuli. One possible reason for the discrepancy between these earlier studies and our results is that the membrane potentials and input resistances of taste cells in the previous studies were very low, probably owing to microelectrode damage. Under these conditions, input resistance is not a reliable measure of the effect of taste stimuli on membrane properties.

Voltage-dependent channels have been found in other sensory receptors including cochlear hair cells (Lewis and Hudspeth, 1983; Fuchs and Mann, 1986) and photoreceptor rod inner segments (Bader et al., 1982; Corey et al., 1984). In those receptors, the voltage-gated channels do not contribute to transduction directly, but act to modulate the receptor potential. In taste cells, our data suggest that at least one voltage-dependent channel, a K⁺ channel, is directly involved in transduction. It will be interesting to determine whether voltage-dependent conductances are involved in the transduction of other, non-ionic taste stimuli.

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