

Voltage-dependent Ionic Currents in Taste Receptor Cells of the Larval Tiger Salamander

KUMIKO SUGIMOTO and JOHN H. TEETER

From the Monell Chemical Senses Center and the Department of Physiology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

ABSTRACT Voltage-dependent membrane currents of cells dissociated from tongues of larval tiger salamanders (*Ambystoma tigrinum*) were studied using whole-cell and single-channel patch-clamp techniques. Nongustatory epithelial cells displayed only passive membrane properties. Cells dissociated from taste buds, presumed to be gustatory receptor cells, generated both inward and outward currents in response to depolarizing voltage steps from a holding potential of -60 or -80 mV. Almost all taste cells displayed a transient inward current that activated at -30 mV, reached a peak between 0 and $+10$ mV and rapidly inactivated. This inward current was blocked by tetrodotoxin (TTX) or by substitution of choline for Na^+ in the bath solution, indicating that it was a Na^+ current. Approximately 60% of the taste cells also displayed a sustained inward current which activated slowly at about -30 mV and reached a peak at 0 to $+10$ mV. The amplitude of the slow inward current was larger when Ca^{2+} was replaced by Ba^{2+} and it was blocked by bath applied Co^{2+} , indicating it was a Ca^{2+} current. Delayed outward K^+ currents were observed in all taste cells although in about 10% of the cells, they were small and activated only at voltages more depolarized than $+10$ mV. Normally, K^+ currents activated at -40 mV and usually showed some inactivation during a 25-ms voltage step. The inactivating component of outward current was not observed at holding potentials more depolarized than -40 mV. The outward currents were blocked by tetraethylammonium chloride (TEA) and BaCl_2 in the bath or by substitution of Cs^+ for K^+ in the pipette solution. Both transient and noninactivating components of outward current were partially suppressed by Co^{2+} , suggesting the presence of a Ca^{2+} -activated K^+ current component. Single-channel currents were recorded in cell-attached and outside-out patches of taste cell membranes. Two types of K^+ channels were partially characterized, one having a mean unitary conductance of 21 pS, and the other, a conductance of 148 pS. These experiments demonstrate that tiger salamander taste cells have a variety of voltage- and ion-dependent currents including Na^+ currents, Ca^{2+} currents and three types of K^+ currents. One or more of these conductances may be modulated either directly by taste stimuli or indirectly by stimulus-regulated

Address reprint requests to Dr. John H. Teeter, Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104. Dr. Sugimoto's permanent address is Department of Physiology, Tokyo Medical and Dental University, 5-45 Yushima 1-chome, Bunkyo-ku, Tokyo 113, Japan.

second messenger systems to give rise to stimulus-activated receptor potentials. Others may play a role in modulation of neurotransmitter release at synapses with taste nerve fibers. Although solitary tiger salamander taste cells, as well as taste cells in isolated pieces of lingual epithelium are capable of discharging action potentials in response to depolarizing currents, the role of action potentials in taste signal transduction or in activation of neurotransmitter release remains unclear.

INTRODUCTION

Taste transduction in vertebrates is mediated by specialized epithelial receptor cells, which are usually grouped into discrete intraepithelial structures, the taste buds (for review, see Kinnamon, 1987). Interaction of chemical stimuli with sites on the exposed, apical membranes of the taste cells is believed to initiate a change in membrane conductance, resulting in depolarization of the cell and release of neurotransmitter at synapses with gustatory nerve fibers (for reviews, see Teeter et al., 1987; Teeter and Brand, 1987; Kinnamon, 1988; Avenet and Lindemann, 1989; Roper, 1989; Teeter and Cagan, 1989). Although depolarizing receptor potentials have been recorded from taste cells in a variety of species, including catfish (Teeter and Brand, 1987), frogs (Sato, 1972; Sato and Beidler, 1975; Akaike et al., 1976; Miyamoto et al., 1988a), mudpuppies (Kinnamon and Roper, 1987, 1988; West and Bernard, 1978), mice (Tonosaki and Funakoshi, 1984), and rats (Ozeki, 1971; Sato and Beidler, 1982, 1983), the nature of the initial stimulus-receptor cell interaction, as well as the subsequent events leading to neurotransmitter release, remain incompletely characterized.

Elucidation of the mechanisms of taste transduction has been difficult for several reasons. Not only are taste cells typically small and tightly packed together in the taste buds, thereby making stable intracellular recordings difficult, but the receptive (apical) surface constitutes only a few percent of the taste cell membrane, so that selective stimulation or recording from this region, even with isolated cells, is problematic (but see Kinnamon et al., 1989). Until recently, taste cells were believed to be electrically inexcitable, responding to chemical stimuli with slow changes in membrane potential, graded in amplitude with stimulus concentration (cf. Sato, 1980; Teeter et al., 1987). However, studies employing improved intracellular recording techniques with large taste cells in *Necturus* (Roper, 1983; Kinnamon and Roper, 1987; Avenet and Lindemann, 1987a), as well as recent whole-cell patch-clamp recordings (Avenet et al., 1988; Avenet and Lindemann, 1987b; Akabas et al., 1988; Kinnamon and Roper, 1988; Miyamoto et al., 1988b; Spielman et al., 1989) have shown not only that taste cells possess a variety of voltage-dependent conductances, but that they are capable of generating action potentials under appropriate conditions. However, the role of regenerative responses in taste transduction remains unclear.

In addition, multiple transduction pathways appear to mediate the responses of taste cells to at least some of the diverse categories of taste stimuli, e.g., salts, acids, sugars, and amino acids. A variety of studies indicate that taste cell responses to Na^+ and Li^+ salts result from direct influx of stimulus cations through voltage-insensitive, amiloride-blockable channels in the apical membrane (Schiffman et al., 1983; DeSimone et al., 1984; Heck et al., 1984; Brand et al., 1985; Avenet and Linde-

mann, 1988). Sour taste, which is produced by acids, appears to be mediated by a transient proton block of a voltage-dependent K^+ conductance in the apical membranes of taste cells (Sugimoto and Teeter, 1987*b*; Kinnamon and Roper, 1988; Kinnamon et al., 1989). Specific membrane receptor proteins have been implicated in taste cell responses to amino acids (e.g., Cagan, 1981, 1986), sugars (Jakimovich, 1982; Faurion, 1987), and some bitter compounds (Akabas et al., 1988). Both receptor-regulated channels (Teeter et al., 1990) and G protein-coupled, stimulus-regulated second messengers (Lancet et al., 1987; Akabas et al., 1988; Kalinoski et al., 1989; Striem et al., 1989) appear to be involved.

We have used whole-cell and excised patch tight-seal recording techniques (Hamill et al., 1981) to investigate the electrical properties of taste cells isolated from the lingual epithelium of larval tiger salamanders. In this report, we present an analysis of the voltage-dependent ionic currents in salamander taste cells. Preliminary reports of this work have been presented (Sugimoto and Teeter, 1987*a, b*).

METHODS

Preparation

Larval (aquatic phase) tiger salamanders (*Ambystoma tigrinum*) were obtained from commercial suppliers and maintained at 10°C in large, biologically filtered holding tanks. They were fed liver and meal worms weekly.

Salamanders were decapitated and pithed and the lingual epithelium rapidly removed from the anterodorsal surface of the tongue by blunt dissection. For intracellular recordings from in situ taste cells, pieces of epithelium were stretched over a glass window in the Sylgard-covered bottom of a recording chamber and fixed in position with pins. For dissociation of solitary taste cells, the epithelium was pinned to the Sylgard-covered bottom of a 30-mm plastic culture dish and incubated in amphibian saline containing 0.4–0.5 mg/ml collagenase and 0.5 mg/ml hyaluronidase (Boehringer Mannheim Biochemicals, Indianapolis, IN) with continuous agitation at room temperature for 20 min. After about 20 min, the epithelium began to separate from the underlying tissue. The enzyme solution was then replaced with Ca^{2+} , Mg^{2+} -free saline containing 2 mM EGTA. After 3–4 min, the surface cell layer completely detached from the underlying connective tissue and only taste bud cells remained on the basement membrane. Taste cells were collected in fire-polished glass pipettes and plated in 30-mm plastic culture dishes that had been coated with Cell-Tak (BioPolymers, Inc., Farmington, CT). The cells which did not adhere to the bottom were washed out. Non-gustatory surface epithelial cells, dissociated by the same procedure, were used in some experiments.

Solutions

The compositions of the extracellular and intracellular solutions are listed in Table I. All solutions were adjusted to pH 7.2–7.4 with either KOH or NaOH and filtered (0.28 μ m, Gelman Sciences, Inc., Ann Arbor, MI), just before use. The majority of experiments were performed in standard external bath solution and with a 110 mM KCl solution containing 10^{-7} M free Ca^{2+} in the pipette. Drugs were dissolved in standard or modified external solution and applied to the bath using either gravity perfusion or a pressure ejection system (Picospritzer, General Valve Corp., Fairfield, NJ).

Electrophysiology

Intracellular micropipettes were pulled from Omegadot capillary tubing (Frederick Haer Inc., Brunswick, ME) and had tip resistances of 80–200 M Ω in Ringer solution. Patch pipettes were fabricated from hematocrit-capillary tubes (Blu-Tip, Fisher Scientific Co., Philadelphia, PA), using a two-stage horizontal puller. Pipette tips were fire-polished and usually coated with Sylgard (Dow Corning Corp., Midland, MI). Unfilled patch pipettes had bubble numbers in methanol of 2.5–3.5 (Corey and Stevens, 1982) and resistances of 2–15 M Ω when filled with normal intracellular solution.

The intracellular recording chamber was mounted to the fixed stage of a microscope (model ACM, Carl Zeiss, Inc., Thornwood, NY) equipped with differential interference contrast optics. Taste bud cells were impaled under direct visual control at $\times 400$ – 800 . The culture dish containing isolated taste cells was attached to the stage of a Diaphot inverted microscope (Nikon Inc., Garden City, NY) modified for Hoffman modulation contrast. Cells were viewed at $\times 300$ – 400 . Gigaseals, ranging from 2 to 12 G Ω , were obtained by applying weak negative pressure to the pipette. The whole-cell recording configuration was obtained by applying

TABLE 1
Composition of Solutions

External solution	Na	K	Ca	Choline	Ba	Co	Cl	TEA	HEPES	EGTA	Glucose
							<i>mM</i>				
Standard	111	3	5	—	—	—	124	—	3	—	2
Enzyme	118	3	2.5	—	—	—	126	—	3	—	—
Ca ²⁺ -free	115	3	—	—	—	—	118	—	10	2	—
Na ⁺ -free	—	3	5	112	—	—	125	—	3	—	—
TEA + Ba ²⁺	107	3	—	—	5	—	125	5	3	—	2
Co ²⁺	111	3	2	—	—	3	124	—	3	—	2
Internal solution	K	Ca	Mg	Cs	Cl	HEPES	EGTA	ATP			
							<i>mM</i>				
Standard	110	1	2	—	116	10	2	2			
Cs ⁺	—	1	2	110	116	10	2	2			

additional suction and/or voltage pulses to rupture the membrane with the pipette held at -60 mV. Outside-out patches were obtained by pulling the pipette away from the cell once the whole-cell configuration had been established.

The membrane voltage signal from intracellular microelectrodes was recorded using a unity-gain electrometer (Neuroprobe 1600, A-M Systems, Everett, WA) with capacitance neutralization and an active bridge circuit for applying constant current pulses. Membrane current and voltage were measured in isolated cells and cell-free patches using the whole-cell and single-channel configurations of the patch-clamp technique (Hamill et al., 1981) with either an Axopatch 1B amplifier (Axon Instruments, Inc., Burlingame, CA) or a LIST EPC-7 amplifier (Medical Systems Corp., Greenvale, NY). The current signal was filtered at 3 kHz, digitized at 20–40 kHz, and stored on an AT microcomputer running pCLAMP software (Axon Instruments, Inc.). Once the whole-cell configuration was obtained, a hyperpolarizing DC potential was applied until no net current was observed. This potential relative to the bath was taken as an estimate of the resting membrane potential of the cell. The cell was then further hyperpolarized to a -60 mV or -80 mV holding potential from which various pulse protocols were applied. Cell capacitance was read directly from the amplifier. The series

resistance of the pipette was estimated by dividing the amplitude of the voltage step by the peak capacitive transient and ranged from 3 to 20 M Ω . Both membrane capacitance and series resistance were compensated electronically. Typical series resistance values after compensation were <10 M Ω compared with an input resistance of 1–6 G Ω . For a peak inward current of 1 nA, this would result in an error of <10 mV.

Membrane surface area was calculated from the measured diameter of isolated taste cells which had become rounded and from cell capacitance estimates, assuming a specific membrane capacitance of 1 μ F/cm², for cells with more complex morphologies. Linear capacitive and leak currents generated by a series of hyperpolarizing voltage pulses applied from the holding potential were subtracted from all records unless otherwise noted. The currents elicited by hyperpolarizing voltage steps displayed no time-dependent components.

Single-channel recordings were made in the cell-attached and outside-out configurations with a 50-G Ω feedback resistor. Idealized records were constructed using pCLAMP. Open time, closed time, and amplitude histograms were generated from the idealized records.

RESULTS

Cell Types

Taste receptor cells in the tiger salamander are grouped into intraepithelial taste buds (Fig. 1 A), which have the basic structural features common to most vertebrates (c.f., Farbman, 1965; Graziadei, 1969; Kinnamon, 1987). The taste buds are associated with low papillae, principally on the dorsal and anterolateral surfaces of the tongue. Ultrastructural studies of tiger salamander taste buds have not been reported. However, on the basis of light microscopic sections, they appear to be very similar to taste buds in the closely related axolotl, *Ambystoma mexicanum* (Fährmann, 1967) and except for their somewhat smaller size, to those in the extensively studied mudpuppy, *Necturus maculosus* (Farbman and Yonkers, 1971; Cummings et al., 1987; Delay and Roper, 1987). Larval tiger salamander taste buds contained elongated light and dark cells, as well as basal cells. Cells dissociated from taste buds of larval tiger salamanders were 12–18 μ m ($15.2 \pm 1.4 \mu$ m; mean \pm SD, $n = 69$) in diameter, while those from mudpuppy taste buds were 25–30 μ m in diameter (Kinnamon and Roper, 1987).

Nongustatory surface epithelial cells dissociated from the lingual epithelium were rounded, measuring from 18 to 35 μ m in diameter, and often occurred in pairs or small clusters (Fig. 1 B). Cells dissociated from taste buds displayed a variety of morphologies ranging from spindle-shaped cells (Fig. 1 C) to spherical cells (Fig. 1 D). With our dissociation procedure, nearly all taste cells became rounded within 20 min after isolation. We could not distinguish between light and dark cells, nor was a distinct population of small, spherical cells observed, similar to that tentatively identified as basal cells in mudpuppies (Kinnamon and Roper, 1988). Two populations of taste bud cells were distinguished on the basis of the relative amplitudes of outward and inward currents, but we were unable to correlate these apparent functional differences with distinct morphological classes of cells.

Passive Properties

The passive membrane properties of in situ and isolated taste cells and nongustatory surface epithelial cells are summarized in Table II. Membrane potentials of taste cells

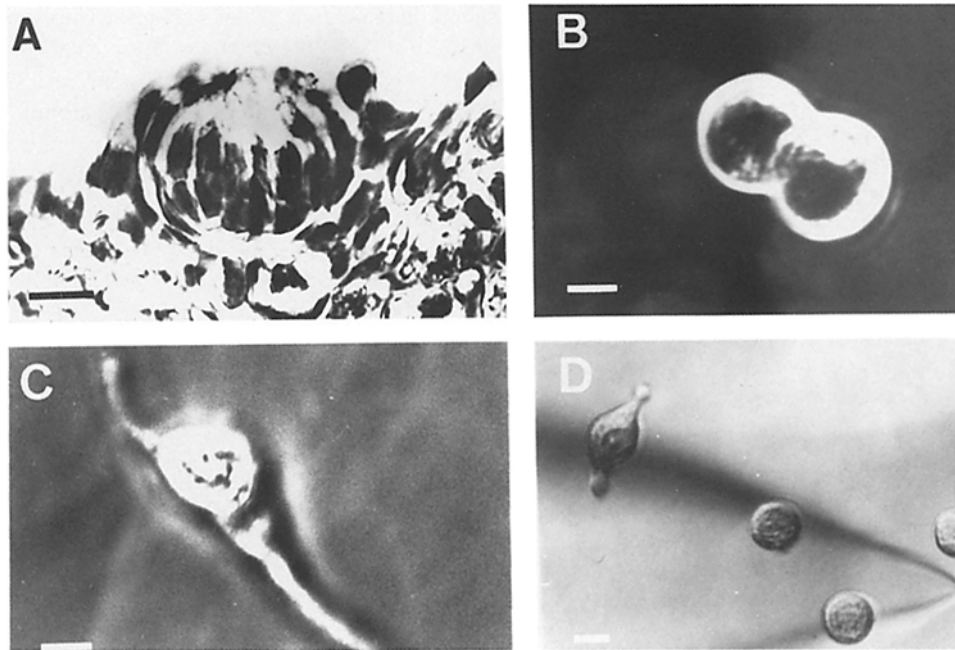


FIGURE 1. Photomicrographs of a taste bud and cells dissociated from lingual epithelia of larval tiger salamanders. (A) Section through a taste bud showing elongated light and dark cells. Frozen section stained with hematoxylin and eosin. Scale bar, 25 μm . A pair of nongustatory epithelial cells (B) and a solitary taste receptor cell (C) dissociated from the lingual epithelium. Phase-contrast. (D) Taste cell in the process of becoming round and four rounded taste cells. Hoffman modulation contrast. Scale bars in B, C, and D, 10 μm .

TABLE II
Passive Membrane Properties of Lingual Cells

Preparation and cell type	Resting potential	Input resistance	Cell capacitance	Surface area
	<i>mV</i>	<i>GΩ</i>	<i>pF</i>	μm^2
Intact epithelium				
Epithelial cell	-30.3 ± 18.2 (7)	0.045 ± 0.029 (7)	—	—
Taste cell	-54.4 ± 14.1 (9)	0.285 ± 0.166 (9)	—	—
Dissociated epithelium				
Epithelial cell	-24.5 ± 7.3 (5)	1.2 ± 0.9 (5)	15.2 ± 9.4 (5)	$1,356 \pm 900$ (10)
Taste cell	-54.8 ± 7.2 (69)	2.5 ± 1.1 (69)	7.4 ± 1.3 (33)	782 ± 140 (69)

Values are expressed as the mean \pm SD with the number of cells in parentheses. The resting potentials in isolated cells were measured either under current clamp or as the zero-current potential under voltage clamp. Input resistance was measured at a holding potential of -60 mV. Values for cell capacitance were read directly from the amplifier. Surface areas of rounded cells were calculated from measured diameters. Surface areas of taste cells with more complex morphologies were calculated using a specific membrane capacitance of $1 \mu\text{F}/\text{cm}^2$. All taste cell values are significantly different from nongustatory cell values ($P < 0.01$).

in isolated pieces of epithelium ranged from -38 to -70 mV, averaging -54 mV in nine cells. Taste cell input resistance varied from 110 to 355 M Ω with an average value of 285 M Ω ($n = 9$). Nongustatory surface cells displayed significantly lower membrane potentials and input resistances, averaging -30 mV and 45 M Ω , respectively ($n = 7$). Membrane potential and input resistance values for isolated taste cells, measured immediately after the whole-cell recording configuration was established, varied from -40 to -76 mV and from 0.8 to 6 G Ω , respectively. The resting potentials in isolated taste cells, measured either under current clamp or as the zero-current potential under voltage clamp did not differ from those recorded from in situ taste cells, averaging -55 mV ($n = 69$). However, the input resistance measured with the patch-clamp technique (2.5 ± 1.1 G Ω , $n = 69$) were considerably larger than those obtained from taste cells using intracellular microelectrodes. This difference is attributable, at least in part, to current leakage around the site of microelectrode penetration. Electrical coupling between some taste cells (Teeter, 1985; Yang and Roper, 1987) may also have contributed to the lower input resistance of cells in situ. Although membrane time constants were not examined in detail, the very slow charging curves observed in current-clamped cells in response to hyperpolarizing current steps (Fig. 2, *B* and *C*), indicated time constants in excess of 100 ms in some isolated taste cells.

Action Potentials in Taste Cells

Taste cells in intact pieces of lingual epithelium often generated a single action potential in response to brief depolarizing current or upon termination of strong hyperpolarizing currents (Fig. 2 *A*). Occasionally, a second or even third, attenuated spike was observed. These action potentials occasionally overshoot 0 mV, but not by more than 5 mV. Under current clamp, isolated taste cells also responded to depolarizing currents with an action potential (Fig. 2 *B*). Even sustained currents usually elicited only a single spike. However, in 5 of 13 cells displaying action potentials, two to five impulses of decreasing amplitude were recorded (Fig. 2 *C*). The threshold for action potential generation was between -22 and -36 mV. The large afterpotentials observed in taste cells in intact pieces of epithelium (Fig. 2 *A*) were not observed in isolated taste cells.

Membrane Currents

Time- and voltage-dependent inward and outward membrane currents were observed after step depolarization of voltage-clamped taste cells. Typical current records from a solitary taste cell held at -60 mV and depolarized in 10 mV steps to $+60$ mV are presented in Fig. 3 *A*. Most taste cells (88%) displayed a transient inward current that activated and inactivated rapidly with depolarizing pulses above -30 to -20 mV. The currents peaked at 0 to $+10$ mV with a mean latency of 0.69 ± 0.16 ms (SD, $n = 69$). These currents were blocked by 1 μ M tetrodotoxin (TTX) or by replacement of the Na⁺ in the bath with choline (Fig. 4), indicating that they were carried by Na⁺. The prominent delayed outward currents observed in most (90%) taste cells activated at -40 to -30 mV and usually displayed both transient and noninactivating components (Fig. 3). Outward currents were blocked either by

replacement of KCl in the pipette with CsCl (Fig. 5, *A* and *B*) or addition of 5 mM tetraethylammonium chloride (TEA) and 5 mM BaCl₂ to the bath (Fig. 5 *C*), indicating that they were carried by K⁺. Cells displaying small outward currents (10% of the cells) or in which outward currents were suppressed by TEA and BaCl₂, often

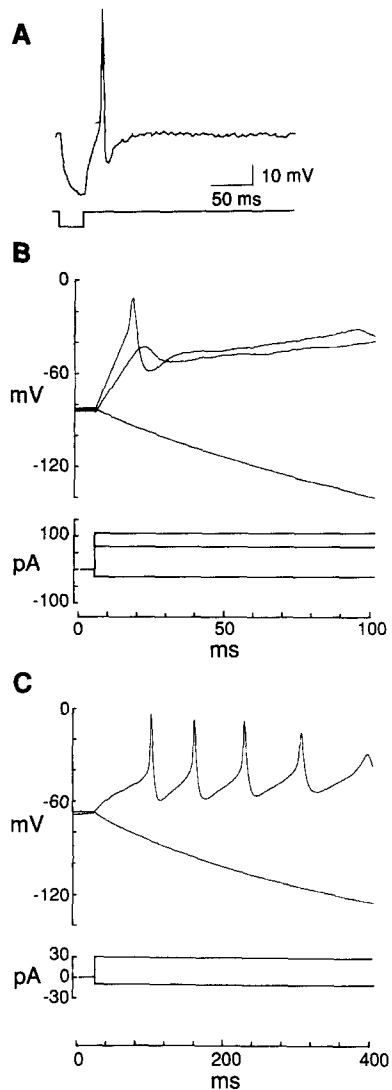


FIGURE 2. Membrane potential recordings from taste cells. (A) A single action potential was discharged upon termination of a hyperpolarizing current pulse in a taste cell in an isolated piece of lingual epithelium. The action potential overshoot 0 mV by 5 mV and had a prominent afterpotential. Resting potential -50 mV. Standard external solution with 5 mM CaCl₂. (B) Isolated taste cells usually discharged a single action potential in response to maintained depolarizing currents (*lower traces*). Hyperpolarizing current steps produced slow electrotonic potentials. Resting potential -82 mV. (C) An isolated taste cell showing repetitive firing in response to a depolarizing current step. Resting potential -67 mV. The bath contained normal external solution in each experiment. Standard pipette solution in *B* and *C*.

displayed slowly activating, sustained inward currents that were blocked by addition of 2 mM Co²⁺ to the bath. These currents were identified as Ca²⁺ currents.

Na⁺ Currents

Voltage-dependent sodium currents were present in most of the taste cells examined, although they varied considerably in amplitude from cell to cell. At a holding

potential of -80 mV, Na^+ -currents ranged from -0.2 to -2.6 nA, while at -60 mV, they varied from -0.1 to -1.7 nA (-0.74 ± 0.39 pA; mean \pm SD, $n = 69$). Na^+ currents were studied in cells which displayed negligible Ca^{2+} currents and in which outward currents had been blocked either by replacing the K^+ in the pipette with Cs^+ or by adding 5 mM TEA and 5 mM BaCl_2 to the bath (Fig. 5). Although the large uncompensated series resistance of our recording system precluded a detailed study of the Na^+ currents, these currents displayed the characteristic activation-inactivation sequence observed in other electrically excitable cells (for review, see Hille, 1984). A typical recording made from a cell displaying negligible Ca^{2+} currents

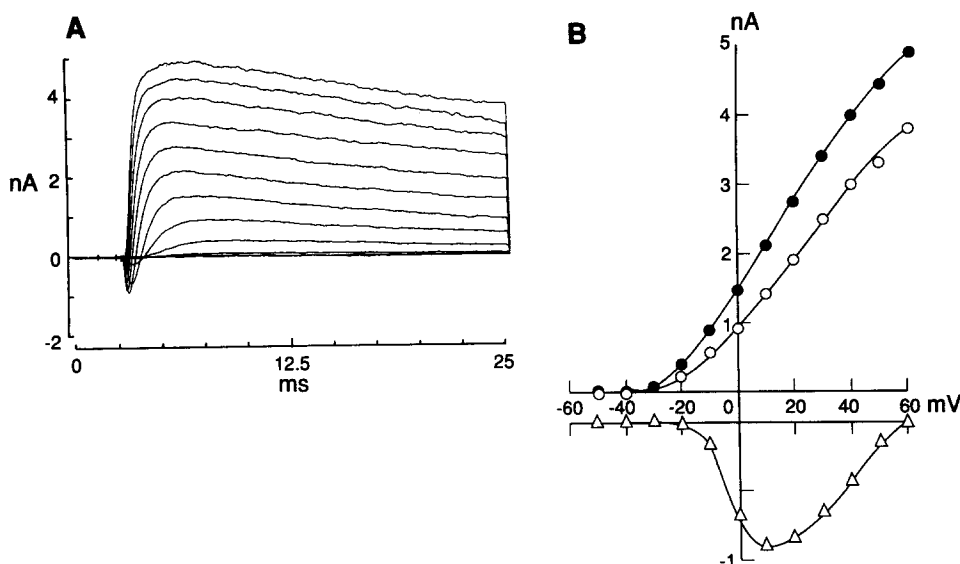


FIGURE 3. Whole-cell currents and the current-voltage relationship from an isolated taste receptor cell. (A) Inward and outward currents elicited by a sequence of pulses between -50 and $+60$ mV, in 10-mV increments, from a holding potential of -60 mV. Outward currents shown as upward deflections. Capacitive and leakage currents were subtracted in this and subsequent figures, except where noted. Standard internal and external solutions. (B) Current-voltage relationship for the cell in A. (Δ) Peak inward currents at each potential; (\bullet) peak outward currents; (\circ) "steady-state" outward currents measured at the end of the voltage pulses.

and with Cs^+ in the pipette is illustrated in Fig. 6 A. The membrane was held at -80 mV and a series of depolarizing voltage steps was applied in 10 mV increments from -70 to $+60$ mV. A pulse, for example, to 0 mV elicited an inward current that reached a maximum of -2.6 pA in about 1 ms and was almost completely inactivated within 3 ms (Fig. 6 A). The current-voltage curve for this cell is presented in Fig. 6 B and shows that the Na^+ current activated between -50 and -40 mV, reached a peak at about 0 mV and appeared to reverse at about $+65$ mV.

The inactivation of the Na^+ current was voltage-dependent. A steady-state inactivation curve for the Na^+ current, generated by plotting normalized peak Na^+

current as a function of holding potential is shown in Fig. 6 C. Inactivation was half-maximal at about -60 mV and the currents were completely blocked at holding potentials positive to -20 mV. This indicates that about half of the Na^+ current is inactivated at the normal resting potential of the cell.

Ca^{2+} Currents

A slowly activating, sustained component of inward current was often observed in cells which displayed negligible outward currents or in which outward currents had

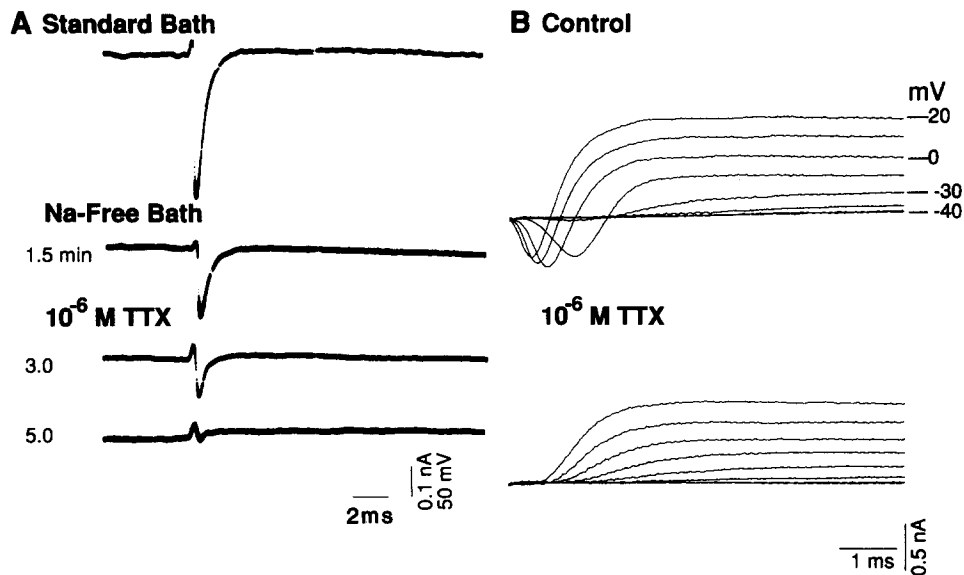


FIGURE 4. Transient inward currents were carried by Na^+ . (A) In the standard bath solution, depolarizing steps from a holding potential of -60 mV to 0 mV elicited a transient inward current. Outward currents were blocked by replacement of K^+ with Cs^+ in the pipette solution. Replacement of the standard external solution with Na^+ -free solution in which Na^+ had been replaced with choline, resulted in a gradual block of the transient inward current. Capacitative and leakage currents were not subtracted in this record. Complete exchange of the bath solution required 5–6 min. (B) The transient inward currents elicited by depolarizing steps to the potentials listed next to each trace were rapidly blocked by addition of $1 \mu\text{M}$ TTX to the bath. Standard external and internal solutions.

been blocked with 5 mM TEA and 5 mM BaCl_2 (Fig. 7). These currents were larger with 5 mM BaCl_2 present (Fig. 7 B) and they were blocked by 2 mM Co^{2+} in the bath (not shown), indicating that they were attributable to Ca^{2+} channels. Fig. 7 C illustrates the current voltage relationships for the Ca^{2+} currents in a cell displaying only small outward currents, both in normal Ringer containing 5 mM CaCl_2 and in Ringer containing 5 mM TEA, 5 mM BaCl_2 , and $1 \mu\text{M}$ TTX. In both bath solutions, the Ca^{2+} currents activated at about -40 mV and reached a peak between 0 and $+10$ mV. However, the peak inward current was nearly three times larger with 5 mM

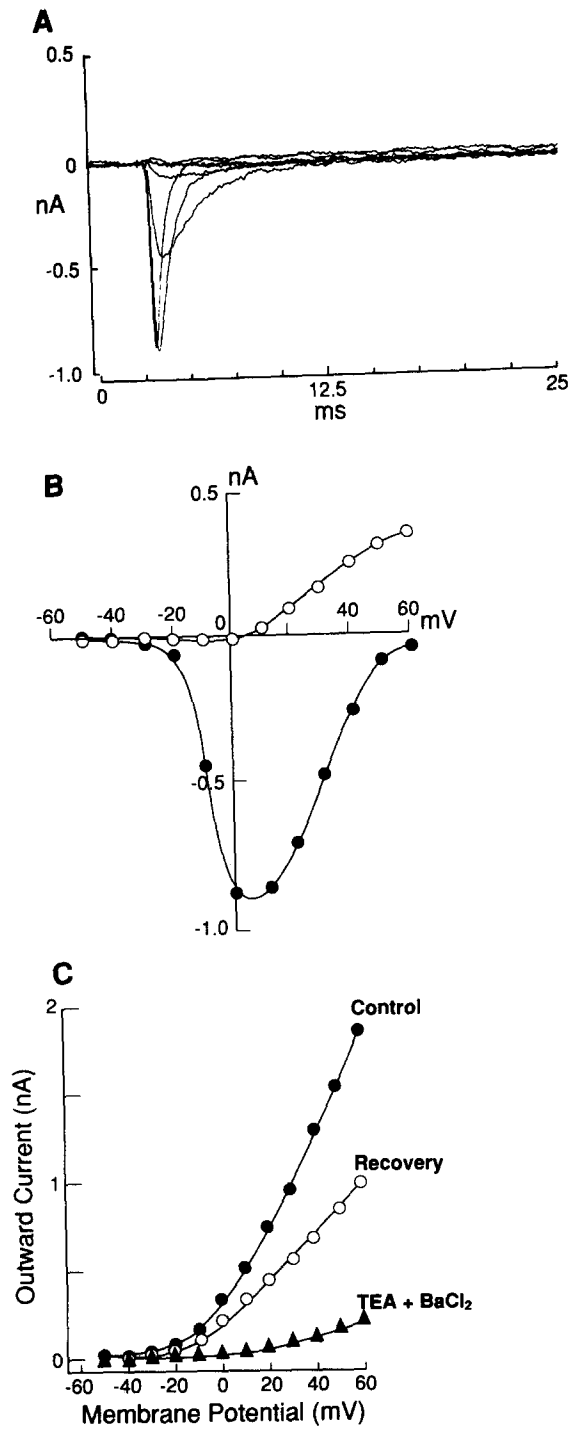


FIGURE 5. Outward currents were carried by K^+ . (A) Substitution of CsCl for KCl in the pipette resulted in nearly complete suppression of voltage-dependent outward currents. (B) Current-voltage relationship for the cell in A. Inward currents (\bullet) were not blocked with CsCl in the pipette, whereas the steady-state outward currents (\circ) were markedly reduced. Compare with Fig. 3. (C) Outward current as a function of test pulse potential before addition of (control), after addition of, and after removal of (recovery) 5 mM TEA and 5 mM BaCl₂ to the bath. Holding potential was -60 mV.

BaCl₂ in the bath than with 5 mM CaCl₂ alone. Ca²⁺ currents were observed in about 60% of the cells examined and varied in amplitude from -50 to -500 pA.

Outward Currents

Voltage-dependent outward currents were a prominent feature of nearly all taste cells examined (cf. Fig. 3). However, a small number of taste cells (10%) displayed

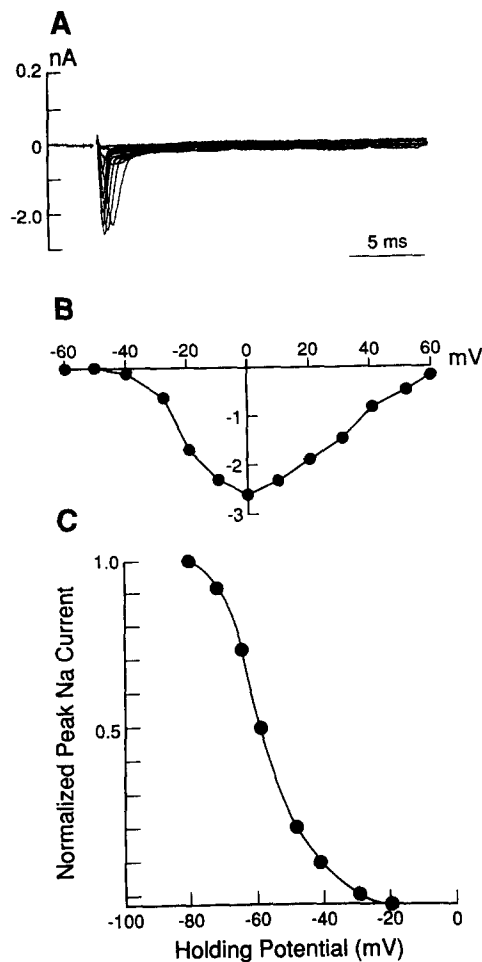


FIGURE 6. Na⁺ currents in a taste cell. (A) The illustrated currents were recorded during voltage steps to potentials between -60 and +60 mV from a holding potential of -80 mV. Outward currents were blocked by replacing K⁺ in the pipette with Cs⁺. (B) Peak Na⁺ currents from the cell in A were plotted as a function of pulse potential. (C) Voltage-dependence of Na⁺-current inactivation in a different cell. Peak currents during a test pulse to 0 mV were normalized and plotted as a function holding potential. Standard external solutions.

only small outward currents, even in response to voltage steps to +60 mV (Fig. 7 A). These cells had normal Na⁺ and Ca²⁺ currents. In cells displaying prominent outward currents, these currents were suppressed, by 70–80% when 5 mM TEA was added to the bath. Addition of 5 mM BaCl₂ to the TEA bath solution further reduced the voltage-dependent outward currents to 0–11% of the controls (*n* = 11) (Fig. 5 C). These results indicate that the outward currents were carried by K⁺.

Outward currents were isolated in Ca^{2+} -free Ringer and with $1 \mu\text{M}$ TTX or a holding potential of -40 mV to inactivate Na^+ currents. The K^+ currents activated within $0.8\text{--}3 \text{ ms}$ of the onset of a depolarizing pulse, reached a peak within $3\text{--}11 \text{ ms}$ and often showed some inactivation within a 25-ms pulse (Fig. 3). The time-dependent decrease in outward current is apparent in the current-voltage curves for peak and steady-state outward currents shown in Fig. 3B. The effect of holding potential on outward current is shown in Fig. 8. The inactivating component of

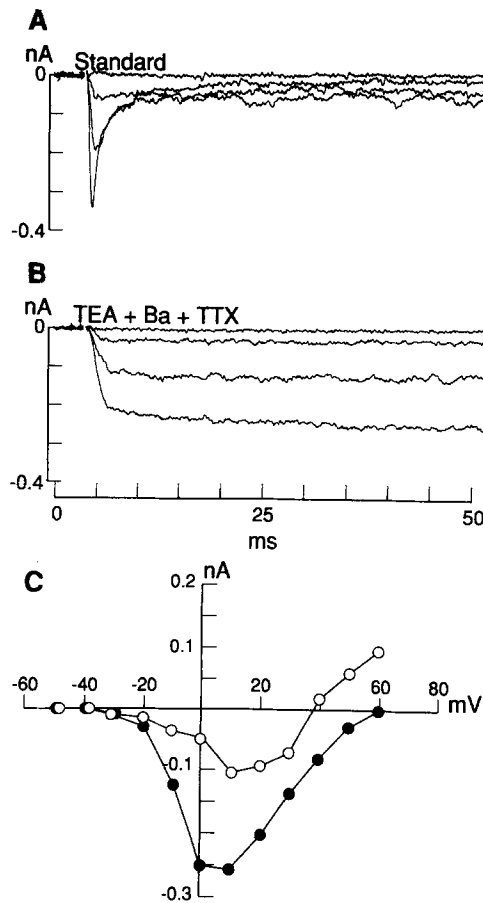


FIGURE 7. Ca^{2+} currents and the Ca^{2+} current-voltage relationship in a taste cell. (A) Inward currents recorded from a taste cell held at -60 mV and stepped to $-40, -20, -10,$ and 0 mV . This cell displayed both transient Na^+ currents and slow, sustained inward currents, but no outward currents in the standard bath solution. (B) The transient Na^+ current was blocked by addition of $1 \mu\text{M}$ TTX to the bath. Addition of 5 mM TEA and 5 mM BaCl_2 resulted in larger, maintained inward currents at the same test potentials used in A. (C) Current-voltage curves for the slow inward currents before (○) and after (●) addition of $1 \mu\text{M}$ TTX, 5 mM TEA, and 5 mM BaCl_2 to the standard bath solution. The cell was held at -60 mV and sequentially stepped to potentials between -50 and $+60 \text{ mV}$ in 10 mV steps.

outward current was reduced in amplitude at holding potentials more depolarized than -60 mV and was completely suppressed at holding potentials positive to -40 mV . Both the rapid rise and slow decline of the outward currents in response to depolarizing pulses above 0 mV were suppressed when the cell was held at -40 mV , with the outward currents continuing to rise slowly during the pulses. Even cells showing no clear inactivation of outward currents during 25-ms pulses displayed some inactivation with longer (150 ms) pulses (not shown). These inactivating K^+

currents resembled the A-currents described in a variety of cells (cf. Adams and Galvan, 1986; Thompson and Aldrich, 1980). The noninactivating component resembled the delayed rectifier current common to many cells (for review, see Hille, 1984). The current-voltage curve for outward current at a holding potential of -40 mV displayed a distinct plateau for depolarizations above $+40$ mV (Fig. 8 B),

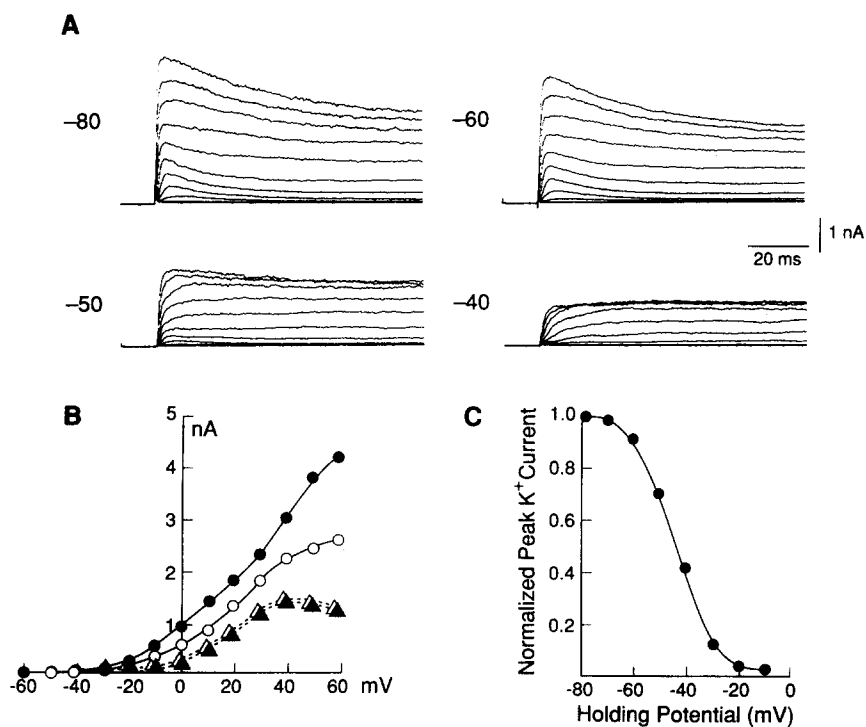


FIGURE 8. Voltage-dependent inactivation of outward currents. (A) Outward currents recorded during voltage pulses to potentials between -50 and $+60$ mV from holding potentials of -80 , -60 , -50 , and -40 mV. Inward currents were negligible in this cell. Capacitive and leakage currents were not subtracted in these records. (B) Current-voltage curve for peak (filled symbols) and steady-state (open symbols) outward currents elicited from holding potentials of -80 mV (circles) and -40 mV (triangles). A plateau is apparent in the curves generated from -40 mV at test potentials greater than $+40$ mV. (C) Steady-state voltage dependence of inactivation of peak outward currents as a function of holding potential in a different cell. Currents were recorded during 50-ms test pulses to $+40$ mV preceded by a 2-s prepulse to the potential indicated on the abscissa. Peak outward currents were normalized to the currents at a holding potential of -80 mV and plotted as a function of prepulse potential. Standard bath solutions.

suggesting that part of the outward current might be Ca^{2+} activated (Meech and Standen, 1975; Adams et al., 1982; Dubinsky and Oxford, 1984; Hudspeth and Lewis, 1988). Outward currents from a taste cell displaying a marked inactivating current component, as well as a clear region of negative slope in the steady-state current-voltage curve, are presented in Fig. 9.

In most cells, addition of 2 mM CoCl_2 to the bath suppressed the voltage-activated steady-state outward currents, but to a variable extent (37–74%). The transient component of outward current was also reduced significantly in some cells (three out of eight cells). The effect of Co^{2+} on a cell displaying marked transient outward currents is shown in Fig. 10 A. Although both the transient and sustained components of current were suppressed by Co^{2+} , about 30% of both currents remained. Current–voltage curves for outward currents in this cell, before and after addition of Co^{2+} to the bath are shown in Fig. 10 B. The negative slope region of the current–voltage curve for steady-state currents was not observed in the presence of CoCl_2 . These results are consistent with the conclusion that a portion of the outward current in the taste cells was activated by Ca^{2+} influx. The results indicate that the

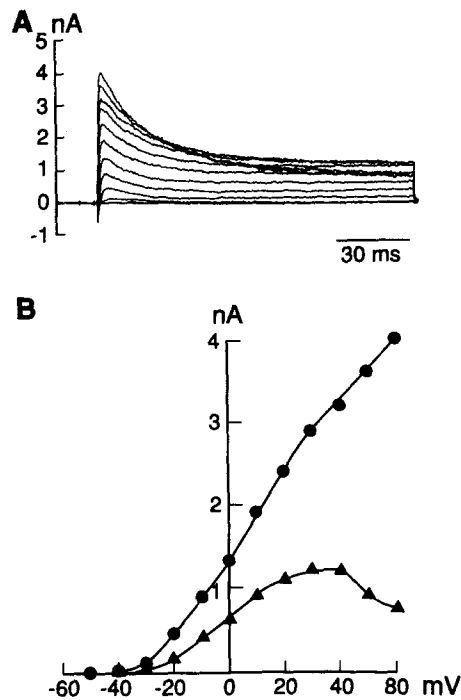


FIGURE 9. Ca^{2+} -dependent component of outward K^+ current. (A) Inward and outward currents during prolonged voltage pulses between -50 and $+60$ mV from a holding potential of -60 mV. The bath contained the standard solution. (B) Current–voltage curves for the transient (●) and steady-state (▲) outward currents for the cell in A. A region of “negative conductance” characteristic of Ca^{2+} -activated K^+ currents is apparent between $+40$ and $+60$ mV.

outward currents in tiger salamander taste cells consisted of at least three components, one attributable to transient, or inactivating K^+ channels (A channels), one to delayed rectifier K^+ channels, and one to calcium-activated K^+ channels. The magnitudes of I_{Ca} , I_{A} , I_{K} , and $I_{\text{K(Ca)}}$ varied greatly from cell to cell making difficult the attempts to relate voltage-dependent Ca^{2+} influx to activation of K^+ currents. Although most taste cells displayed transient, delayed rectifier and Ca^{2+} -activated K^+ currents, some had much more prominent inactivating outward currents than others (cf. Figs. 9 and 10). No clear correlation between the presence or amplitude of transient outward currents and cell appearance or passive membrane properties was observed.

Although variable in magnitude, inwardly rectifying K^+ currents were reported in mudpuppy taste cells (Kinnamon and Roper, 1988). We found no clear evidence for inward rectifier K^+ channels in isolated tiger salamander taste cells.

Single-Channel K^+ Currents

Single-channel currents were recorded from taste cells in the cell-attached configuration and from excised outside-out patches of membrane. Most experiments were done at steady holding potentials and rapidly inactivating currents like those attributable to voltage-dependent Na^+ channels and A channels would not have been observed. Steady-state single-channel currents in an outside-out patch of membrane from a taste cell displaying large whole-cell K^+ currents are presented in Fig. 11. The

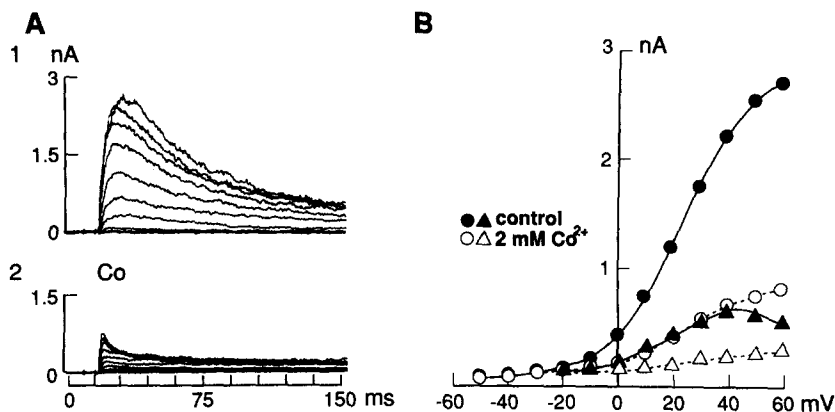


FIGURE 10. Ca^{2+} dependence of outward current. (A) Outward currents during voltage pulses between -50 and $+60$ mV from a holding potential of -60 mV were suppressed by addition of 2 mM $CoCl_2$ to the bath. The marked transient component of outward current in this cell, as well as the steady-state outward current, were reduced by Co^{2+} . (B) Current-voltage relationships for steady-state outward currents in the same cell, before (*open symbols*) and after (*filled symbols*) addition of 2 mM $CoCl_2$ to the bath. The region of negative slope in the curve for steady-state outward currents was absent in the presence of 2 mM $CoCl_2$. The holding potential was -60 mV.

macroscopic outward currents showed only slight inactivation during a 25-ms pulse to $+40$ mV (Fig. 11 A). The excised patch of membrane from this cell contained at least two channels, each with a unitary conductance of 148 pS (Fig. 11 B). The extrapolated reversal potential for these currents was -48 mV in standard internal and external solutions (Fig. 11 C), indicating that the channels were somewhat permeable to ions other than K^+ , which had an equilibrium potential of -90 mV under these conditions. The average slope conductance for large conductance channels in 12 cells was 147.7 ± 6.6 pS (mean \pm SD).

In addition to the large conductance channels, taste cells often displayed smaller single-channel K^+ currents having a slope conductance of 20.5 ± 2.9 pS (mean \pm SD, $n = 6$) (Fig. 12 A). Current-voltage curves for single-channel currents

from eight different outside-out patches of taste cell membrane are shown in Fig. 12 *B*. Two classes of channels, one with unitary conductances of about 148 pS and one with conductances of about 21 pS, are apparent. Both types of channels were frequently recorded from a single patch, indicating similar distributions in the membrane. The currents through the smaller conductance channels had extrapolated reversal potentials near -80 mV, close to the K^+ equilibrium potential of -90 mV. Currents through the large conductance channels appeared to reverse near -50 mV. Both the 148- and 21-pS currents were blocked by addition of 5 mM TEA and 5 mM $BaCl_2$ in the bath, consistent with their identification as K^+ currents. An

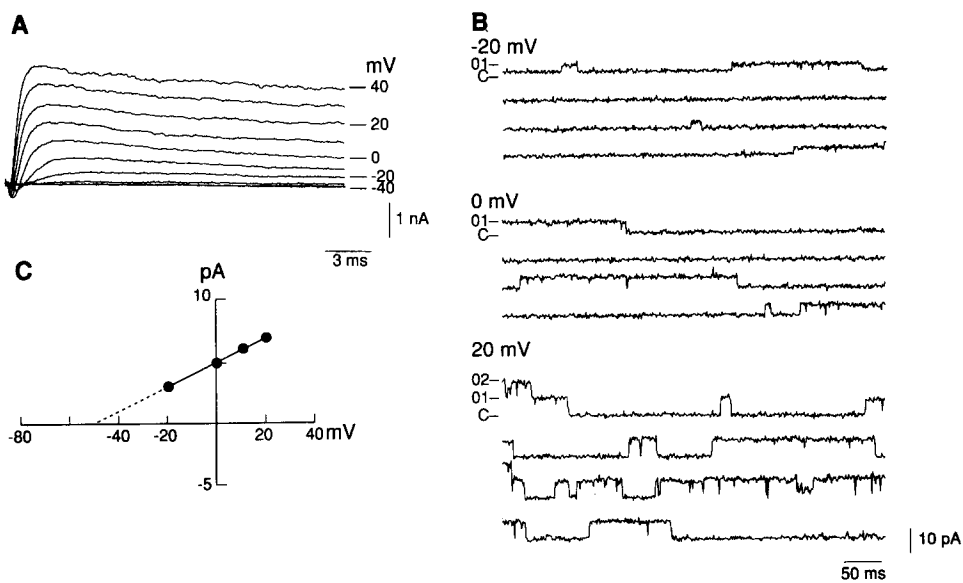


FIGURE 11. Whole-cell and single-channel K^+ currents in a taste cell. (A) Whole-cell currents elicited by voltage pulses to potentials between -50 and $+40$ mV from a holding potential of -60 mV. (B) Single-channel currents at various holding potentials in an outside-out patch from the cell in A. Outward current (channel opening) is upward. Two channels of equal conductance (01, 02) were apparent at $+20$ mV. (C) Steady-state current-voltage curve for current records in B. The slope conductance is 147 pS and the extrapolated reversal potential is about -50 mV, indicating that these are K^+ channels. Standard solutions.

example of the effect of 5 mM TEA and 5 mM $BaCl_2$ on a K^+ channel having unitary conductance of 142 pS is shown in Fig. 12 *C*. Channel block appeared to occur in two steps in this patch.

The distributions of open and closed times for a large conductance channel at -10 mV are presented in Fig. 13, *A* and *B*. The open durations were fitted with the sum of two exponentials with time constants of 1.3 and 14.1 ms (Fig. 13 *A*). The mean open time of this channel increased with depolarization up to 0 mV, but decreased at holding potentials beyond 0 mV. The distribution of closed times was fit by the sum of two exponentials with time constants of 0.7 and 9.5 ms (Fig. 13 *B*).

The small-conductance K^+ channels displayed somewhat different steady-state kinetics. The durations of the opening and closing of a small-conductance channel at -10 mV are presented in Fig. 13, *C* and *D*. The open time distribution was fit by the sum of two exponentials with time constants of 1.8 and 6.5 ms (Fig. 13 *C*). The mean open time increased with depolarization, while the closed times decreased, indicating

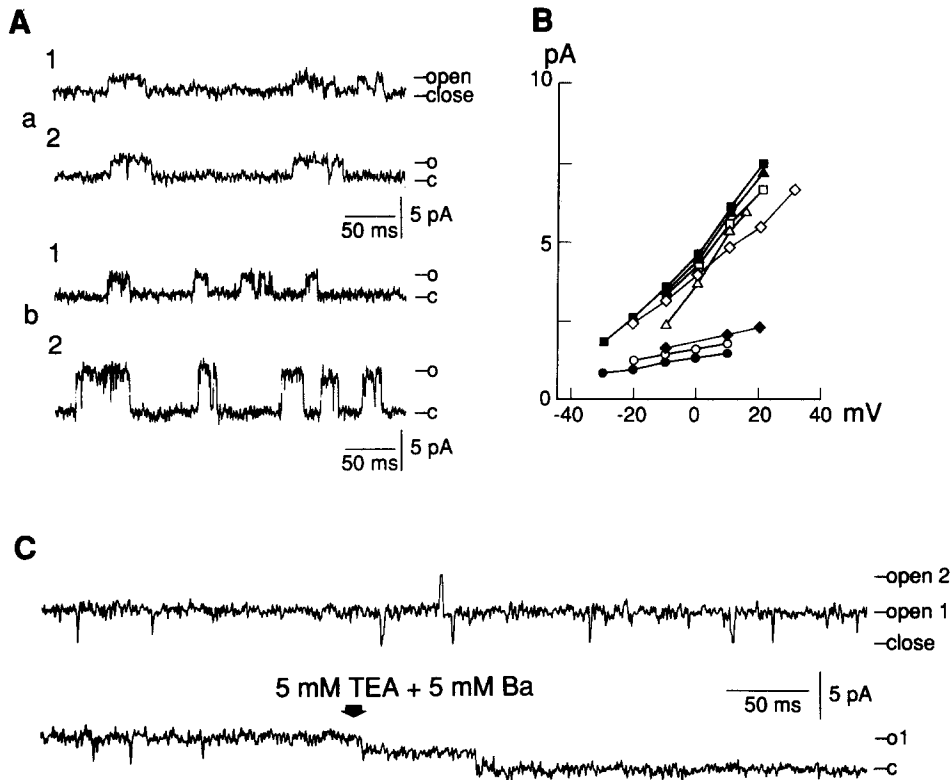


FIGURE 12. Single-channel K^+ currents from outside-out patches of taste cell membrane. (A) Examples of a small conductance (21 pS, *a*) and a large conductance (147 pS, *b*). The pipettes were held at -20 mV (1) and $+10$ mV (2) for each patch. (B) Current-voltage curves for five large-conductance and three low-conductance K^+ channels in membrane patches from different taste cells. (C) K^+ channels were blocked by TEA and $BaCl_2$. Two K^+ channels, each with a conductance of 142 pS, were present in the patch. Addition of 5 mM TEA and 5 mM $BaCl_2$ to the bath resulted in closing of channels.

an increase in open probability. The closed time distribution for this channel was fitted with two exponentials with time constants of 1.5 and 11.5 ms (Fig. 13 *D*).

DISCUSSION

In this study, we have demonstrated that taste receptor cells dissociated from the tongues of larval tiger salamanders display a variety of voltage- and ion-dependent conductances. Solitary taste cells have substantial resting potentials and input

resistances and, like taste cells in isolated pieces of lingual epithelium, are capable of discharging action potentials. The conductances observed in larval tiger salamander taste cells are in many ways similar to those described in taste cells from *Necturus* (Kinnamon and Roper, 1988) and frogs (Avenet and Lindemann, 1987b; Miyamoto

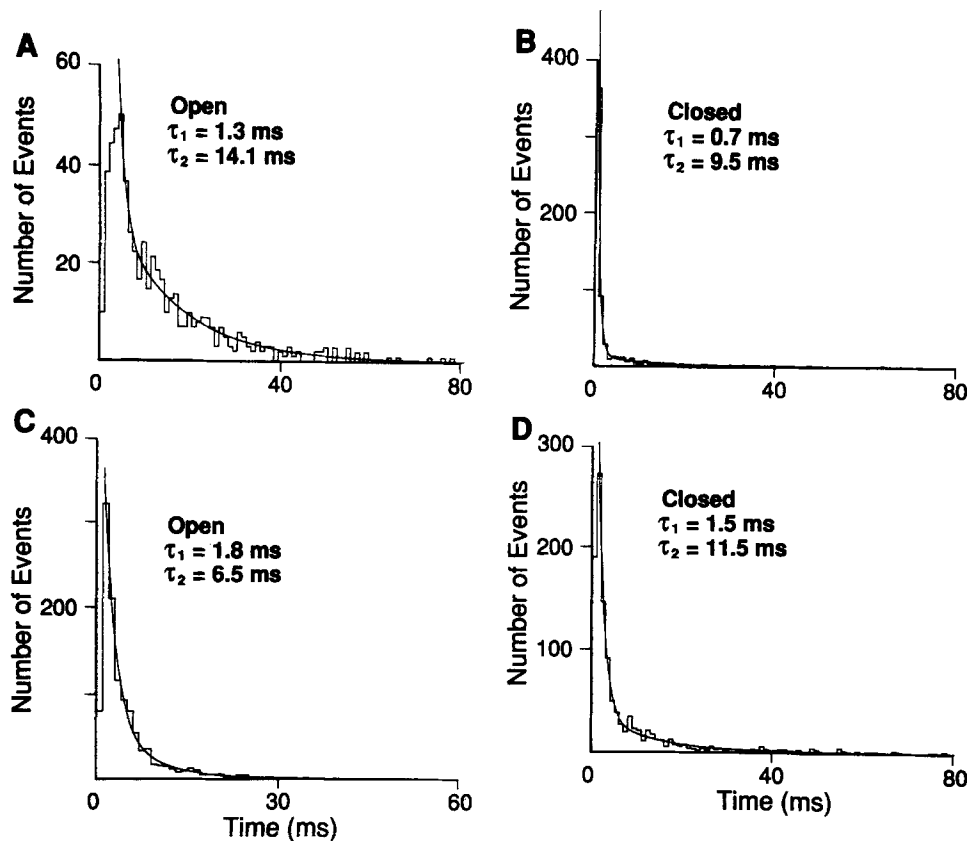


FIGURE 13. Steady-state kinetic properties of the large-conductance (A and B) and small-conductance (C and D) K^+ channels. (A) Histogram of open times at -10 mV for a large-conductance channel. The solid line corresponds to the fit with two exponentials with time constants of 1.3 and 14.1 ms. Total of 581 events in 26 s. (B) Closed time histogram at -10 mV for the single channel as in A. The data were fitted with the sum of two exponentials with time constants of 0.7 and 9.5 ms. (C) Distribution of open times at -10 mV for a small-conductance K^+ channel. The data were fit with two exponentials with time constants of 1.8 and 6.5 ms. Total of 1,196 events in 20 s. (D) Closed time histogram at -10 mV. The distribution was fitted with two exponentials with time constants of 1.5 and 11.5 ms.

et al., 1988b). However, they differ significantly in several respects, including the presence of an inactivating component of outward current (A current) and Ca^{2+} currents that are smaller than reported in *Necturus*. Voltage-dependent Ca^{2+} currents have not been observed in frogs.

The Na^+ currents in tiger salamander taste cells are qualitatively similar to Na^+ currents in a variety of excitable cells (for review, see Hille, 1984). The voltage dependence of peak current and inactivation, as well as the pharmacological properties of these currents, are similar to those reported in taste cells from other amphibians (Avenet and Lindemann, 1987*b*; Kinnamon and Roper, 1988; Miyamoto et al., 1988*b*). Approximately half of the Na^+ current is inactivated at -60 mV, the normal resting potential of the cells, and it is almost completely inactivated at -30 mV. This would explain why regenerative responses have not been observed in taste cells *in vivo*, where resting potentials are typically less than -40 mV (West and Bernard, 1978; Sato, 1980; Sato and Beidler, 1983; Tonosaki and Funakoshi, 1984), probably because of damage resulting from the intracellular microelectrode. Taste cells in *Necturus* (Kinnamon and Roper, 1987, 1988) and frogs (Avenet and Lindemann, 1987*b*; Miyamoto et al., 1988*b*) discharge a single action potential in response to sustained depolarizing currents. This is consistent with the relatively long time constant for recovery from inactivation of Na^+ -currents shown in frog taste cells (Avenet and Lindemann, 1987*b*). In the tiger salamander, trains of two to five action potentials are observed in response to maintained currents in some taste cells, suggesting that recovery from inactivation in these cells was sufficiently rapid to allow repetitive firing. The inactivating component of outward current observed in many tiger salamander taste cells may contribute to the ability of some cells to discharge several action potentials. The slow time course of the action potentials suggest that Ca^{2+} currents are a major component of the regenerative responses.

The Ca^{2+} currents in salamander taste cells have been identified on the bases of their time course, sensitivity to Co^{2+} block, and the ability of Ba^{2+} to substitute for Ca^{2+} as the current carrier. Voltage-dependent Ca^{2+} currents are present in about 60% of the taste cells. The magnitude of the Ca^{2+} current varies greatly from cell to cell, but does not exceed -500 pA. This is considerably smaller than the Ca^{2+} currents observed in *Necturus* taste cells which, although variable in amplitude, could exceed -1.5 nA (Kinnamon and Roper, 1988). The smaller Ca^{2+} currents in salamander taste cell may have resulted, in part, from the smaller size of these cells and, thus, a more rapid "wash out" of the Ca^{2+} currents, which have been shown to decline over time in a variety of internally perfused cells (Kostyuk, 1984). Even with the large variability from cell to cell, there appeared to be a general decrease in the amplitude of the Ca^{2+} current with time, which was slowed, but not prevented, by addition of 2 mM ATP to the pipette solution (Byerly and Yazejian, 1986).

The Ca^{2+} current activates quite rapidly between -40 and -30 mV and does not inactivate during long pulses, suggesting it may contribute significantly to the upstroke of the action potentials in these cells. In contrast, the large Ca^{2+} currents in *Necturus* taste cells activate only with depolarizations above -20 mV and show slow voltage-dependent inactivation (Kinnamon and Roper, 1988), suggesting that they contribute to the plateau of the action potential in taste cells in this species (Roper, 1983; Kinnamon and Roper, 1987). In salamander taste cells, the Ca^{2+} conductance is not activated at the resting potential and it does not inactivate significantly during moderate depolarizations. These properties are consistent both with a role in release of neurotransmitter from taste cells, which is Ca^{2+} dependent (Nagahama and Kurihara, 1985), and with the low levels of spontaneous activity observed in taste

nerve fibers (cf. Frank, 1973), which presumably results from a low rate of transmitter release at rest.

Several different classes of Ca^{2+} channels have been identified and more than one class may be present in a particular cell (Fishman and Spector, 1981; Nowycky et al., 1985; Bean et al., 1986). The sustained Ca^{2+} currents recorded from salamander taste cells displayed no clear inactivation suggesting that they resulted from opening of L-type Ca^{2+} channels. Transient Ca^{2+} currents have been recorded from a variety of cells and have been implicated in repetitive discharge of action potentials (Carbone and Lux, 1984; Nilius et al., 1985). The occurrence of an A-current component suggests that repetitively firing taste cells may also have a transient Ca^{2+} current, which is involved in spacing repetitive responses (for review, see Hille, 1984). Classification of the types of Ca^{2+} channels present in taste cells and their roles in taste reception will require additional studies.

Although the anode-break potentials recorded from *in situ* taste cells in frogs have a Ca^{2+} dependent component (Kashiwayanagi et al., 1983), voltage-dependent Ca^{2+} currents have not been observed in isolated frog taste cells (Avenet and Lindemann, 1987b; Miyamoto et al., 1988b). Wash-out of Ca^{2+} currents may be rapid in these cells, which are somewhat smaller than those from tiger salamanders and considerably smaller than those from *Necturus*.

Outward K^+ currents are a prominent feature of most tiger salamander taste cells and at least three components can be distinguished. One component activates rapidly at depolarizations above -40 mV and displays a variable amount of relatively rapid inactivation. This transient current resembles the A current first described in molluscan neurons (Conner and Stevens, 1971) and subsequently found in a variety of cells (for reviews, see Thompson and Aldrich, 1980; Adams and Galvan, 1986). Unlike A currents in some cells, those in larval salamander taste cells are blocked by TEA and BaCl_2 . Inactivating K^+ currents have not been observed in *Necturus* taste cells (Kinnamon and Roper, 1988). However, slowly inactivating K^+ currents are observed in response to strong depolarizing pulses in some frog taste cells (Avenet and Lindemann, 1987b). The second type of K^+ current is voltage-dependent and resembles the delayed rectifier current observed in a variety of cells. The third type of K^+ current is Ca^{2+} -dependent. This current is also voltage-dependent, activates slowly, and does not inactivate during prolonged voltage steps. The Ca^{2+} -activated K^+ current could account for the large afterpotentials observed in taste cells *in situ*. However, afterpotentials are not observed in isolated taste cells, indicating that some component of this process is inactive. Inwardly rectifying K^+ currents, which are present in isolated *Necturus* taste cells (Kinnamon and Roper, 1988), were not observed in salamander taste cells.

Two distinct types of single-channel K^+ currents were observed in cell-free patch experiments; one having a unitary conductance of about 21 pS and the other, an average conductance of 148 pS. It is tempting to attribute the delayed rectifier component of macroscopic outward currents to the 21 pS class of K^+ channels and the Ca^{2+} -activated component to the 148-pS channels. However, the evidence for this correlation is indirect and based largely on the relative conductances of the two classes of K^+ channels. Neither the kinetics nor Ca^{2+} dependence of the single-channel currents were systematically examined in this study. Other K^+ channel types

may have been present, but not observed under the steady-state conditions of our studies. Rapidly inactivating K^+ -channels would not have been observed with the steady-state voltage protocols used for single-channel recordings.

There was considerable variability in the types and magnitudes of the membrane currents observed in different taste cells. Nearly all taste cells displayed voltage-dependent Na^+ currents, although the peak amplitude varied from cell to cell. Voltage-dependent Ca^{2+} currents were present in about 60% of the taste cells examined and were also variable in amplitude and susceptible to "wash out" over time. The transient and delayed rectifier K^+ currents were present in most taste cells, but their proportions appeared to vary from cell to cell giving rise to a range of whole-cell current patterns. This variability made it difficult to correlate the presence and amplitudes of the various currents, even within a single cell. For example, attempts to relate voltage-dependent Ca^{2+} influx to activation of Ca^{2+} -dependent K^+ currents were unsuccessful.

The variability in currents observed in tiger salamander taste cells may have resulted in part from wash-out or changes in the integrity of membrane conductance resulting from the methods used to dissociate the taste buds. However, some of the differences, particularly whether or not a current was present, may be related to functional differences (perhaps developmental stages) in the cells. Taste cells in all species that have been examined undergo continuous turnover, with older taste cells being replaced by cells differentiating from stem cells in the surrounding epithelium (for review, see Kinnamon, 1987). In the mouse, it has been hypothesized that morphologically distinct dark cells arise from the basal cells and mature into light cells, which form a separate morphological category of cells (Delay et al., 1986). Although this developmental sequence remains to be substantiated, it has been suggested that differences in the type and magnitude of membrane currents in *Necturus* taste cells may correspond to different development stages (Kinnamon and Roper, 1988). Cells believed to be basal cells on the basis of morphological criteria displayed prominent K^+ currents, but little voltage-dependent inward current. Other, presumably more mature, cells displayed large Na^+ , Ca^{2+} , and K^+ currents. Although taste bud cells displaying prominent K^+ currents and small inward currents were observed in the tiger salamander, no correlation with a particular morphological category of cells was apparent. Approximately 10% of the taste cells examined had normal Na^+ and Ca^{2+} currents, but very small K^+ currents. A similar proportion of "inward current dominant" taste cells was reported in studies of frog taste cells (Miyamoto et al., 1988b). However, the significance of this apparent functional difference is unclear. Light cells and dark cells are present in about equal numbers in salamander taste buds and basal cells constitute less than 5% of the taste bud cells.

Recent studies with *Necturus* taste cells (Kinnamon et al., 1989; Roper and McBride, 1989) indicate that voltage-dependent K^+ channels are concentrated in the very small apical surface of the cells, with relatively few K^+ channels being present in the basolateral membrane. Membrane patches excised from tiger salamander taste cells usually displayed one or two types of K^+ channels. These cells were usually rounded and it is highly unlikely that we consistently pulled patches of apical membrane which constitutes only a few percent of the total cell membrane area. However, it is possible that K^+ channels, initially localized in the apical membrane,

were redistributed throughout the membrane during the process of cell rounding. Although *Necturus* taste cells also become rounded in primary culture, they are larger than taste cells from the tiger salamander which may result in slower channel redistribution.

Our studies reveal a variety of macroscopic ionic currents in tiger salamander taste receptor cells that could play a role in taste transduction and subsequent release of neurotransmitter at synapses with taste fibers. The effects of taste stimuli on these voltage-dependent conductances will be described elsewhere.

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