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Identification of Electrophysiologically Distinct Subpopulations of Rat Taste Cells

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Summary. The gustatory sensory system provides animals with a rapid chemical analysis of a potential food substance providing information necessary to facilitate ingestion or rejection of the food. The process of gustatory transduction is initiated in the taste cells in the lingual epithelium. However, due to the small size, scarcity of the cells and their location, embedded in a keratinized squamous epithelium, it has been difficult to study the primary events in the transduction process. Recently, we have developed a preparation of dissociated rat taste cells that permits studies of the taste transduction process in single isolated cells. We have now investigated the electrophysiological properties of the rat taste cells using the patch-clamp technique. We have identified two populations of cells within the taste bud: one expressing a voltage-dependent potassium current and the second containing both voltage-dependent sodium and potassium currents. The potassium current in both cell groups is blocked by external TEA, Ba^{2+}, and quinine. Two types of K^+ channels have been identified: a 90-pS delayed rectifier K^- channel and a "maxi" calcium-activated K^+ channel. The sodium current is blocked by TTX, but not by amiloride.

Key Words taste · gustatory transduction · ion channels · patch clamp · physiology · potassium channels

Introduction

Though the function of the taste buds has been inferred for over 100 years, very little is known about the physiology of the taste cells or the molecular mechanisms involved in the gustatory transduction process (Pfaff, 1985). The structure of the taste buds, which permits the cells to survive in the harsh and variable environment on the surface of the tongue, also makes them relatively inaccessible in situ to direct study. Morphologically, the taste cells are polarized neuroepithelial cells joined to each other by tight junctions (Akisaka & Oda, 1978; Holland, Zampighi & Simon, 1989; Roper, 1989). Their apical surface contains microvilli which project into the taste pore, a small opening to the surface of the tongue through the keratinized squamous cells that surround the taste buds. The microvilli are presumed to be the site of interaction of taste cells with sapid substances. On the basolateral surface the taste cells form chemical synapses with the peripheral terminals of primary gustatory neurons which convey information to the central nervous system (Farbman, 1965; Murray, Murray & Fujimoto, 1969; Kinnamon et al., 1985).

Attempts to study mammalian taste cells in situ with intracellular microelectrodes have been difficult because of their small size and inaccessibility. The low resting membrane potentials, ranging from -10 to -40 mV, reported in most studies of mammalian taste cells (Sato, 1980) suggest that the cells have been damaged at the electrode penetration site. Recently several groups have reported the results of patch-clamp studies of amphibian taste cells (Avenet & Lindemann 1987; Avenet, Hofmann & Lindemann, 1988; Kinnamon & Roper, 1988). These studies have demonstrated the presence of a wide variety of ion channels, including voltage-dependent Na^+ and Ca^{2+} channels. While experiments on amphibian taste cells have provided information about their membrane properties, little is known about the gustatory behavior of these animals, making it difficult to correlate the electrophysiologic studies with the transduction process. Extensive psychophysiologic studies have been conducted to elucidate the perception of taste by rodents (for examples, *see* Pfaff, 1985). Rats respond to a range of substances similar to that detected by humans, and they appear to generalize the taste quality in a similar manner (Frank, 1975; Frank, Contreras & Hettinger, 1983). This suggests that rodents and humans may have similar cell surface receptors, mechanisms of taste transduction and subsequent CNS processing.

In order to study the mechanism of sensory transduction in taste cells, we have developed a procedure to disaggregate the lingual epithelium surrounding the circumvallate papillae of the rat into a dissociated cell suspension. Using this cell preparation, we have shown that a bitter substance,

denatonium, induced a rise in intracellular calcium in a subpopulation of taste cells, due to release of calcium from internal stores (Akabas, Dodd & Al-Awqati, 1988). This suggests that bitter taste transduction involves a cell surface receptor and that ligand binding by this receptor stimulates the formation of an intracellular second messenger. It also suggests that at least some of the functional characteristics of the cells are preserved following dissociation. This report describes results of a patch-clamp study of the rat taste cells in our dissociated cell preparation. Preliminary reports of this work have been published elsewhere (Akabas, Dodd & Al-Awqati, 1987).

Materials and Methods

Preparation of Taste Cells

The procedure to dissociate the rat lingual epithelium into a cell suspension has been described elsewhere (Akabas et al., 1988). Briefly, the tongue was removed from a male Sprague-Dawley rat (200 g) and 0.5 ml of 0.25% collagenase (Sigma) in Hank's Balanced Salt Solution (HBSS) was injected subcutaneously around the circumvallate papillae. The injected tongue was incubated in HBSS at 37°C for 30 min. The epithelium around the papillae was then lifted away from the rest of the tongue and placed in 0.5 ml of 0.25% collagenase, 0.25% trypsin (Cooper) in HBSS and incubated at room temperature for 20 min. All subsequent steps were performed at room temperature. NaEDTA was then added to a final concentration of 4 mM, and the epithelium was triturated 25 times with a fire polished Pasteur pipette. The cells were then incubated for 20 min and then hyaluronidase (Cooper) and neuraminidase (Sigma) were added to final concentrations of 1 mg/ml and 0.1 U/ml, respectively, and the cell suspension was incubated for another 20 min. The suspension was triturated 25 times, 0.2 ml of fetal calf serum (FCS) and 0.5 ml of HBSS were added and the cells pelleted by centrifugation at 100 × *g* for 5 min. They were resuspended in 1 ml of 140 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose, 10 mM NaHEPES at pH 7.4 (buffer A), washed two more times in buffer A and then used for patch-clamp studies.

Cell Labeling Procedures

For experiments with antibody-labeled cells, following dissociation, the cells were resuspended in Lebovitz's L15 medium supplemented with 0.5 mg/ml BSA and 8 mg/ml glucose (L15++). The cells were incubated with a 1:50 dilution of anti-Le^b antibody (Chemibiomed, Alberta, Canada) or a 1:1 dilution of monoclonal antibody 143-2-A6 (ATCC, Maryland) in L15++ plus 10% FCS for 30 min at room temperature. They were washed three times in L15++ and then incubated with a 1:100 dilution of FITC-goat-anti-mouse IgM (Tago) in L15++ plus 1% normal goat serum for 30 min. The cells were washed three times in buffer A and then used for patch-clamp recording. Controls incubated with secondary antibody only showed no labeling. The cells were examined using epifluorescence optics on a Zeiss IM inverted microscope.

Patch-Clamp Recording

For patch-clamp recording the cells were suspended in buffer A and allowed to settle on the bottom of a polystyrene petri dish to which they adhere fairly tightly. Whole-cell and patch recordings were performed by the methods of Hamill et al. (1981). Patch pipettes were pulled in two steps from soda lime glass (Fisher hematocrit tubes) and typically had a resistance of 5 to 10 MΩ after fire-polishing. The electrodes contained (in mM): 140 KCl, 1 $MgCl_2$, 10 KEGTA, 10 NaHEPES at pH 7.2 (buffer E), unless otherwise indicated. Current records were recorded using a List EPC-7 amplifier in the voltage-clamp mode. They were low pass filtered at 1000 Hz, unless otherwise indicated, through an 8-pole Bessel filter (Frequency Devices), digitized at 2 kHz and stored on hard disk. All data acquisition and analysis was performed by digital computer (INDEC Systems, Sunnyvale, CA). Capacitive and linear leak currents have been subtracted from the records.

Results

Dissociation and Identification of Taste Cells

Dissociation of the lingual epithelium lining the circumvallate papillae of the rat, the region with the highest density of taste buds, yielded a suspension which contained mostly single cells and a small number of cell clusters (estimated to be about 50–100/prep) each containing 10–30 cells. Because the taste cells constitute less than 0.1% of the lingual epithelial cells, it was essential to be able to positively identify them in the dissociated cell preparation. We have previously shown that a monoclonal antibody (MAb), which recognizes the Lewis *b* blood group epitope, specifically labels the taste buds in the rat circumvallate papillae (Akabas et al., 1988). Immunofluorescent labeling of the cell suspension with the MAb described above, revealed that the antibody labeled about 50% of the small clusters of cells and rare single cells. Thus, after dissociation the taste buds remained as small clusters of cells, but the remainder of the epithelium dissociated into single cells.

Whole-Cell Recording

We have characterized the electrophysiologic properties of the taste cells in the antibody-labeled clusters using the patch clamp technique. Initially, the dissociation procedure utilized only trypsin and collagenase; the success rate of obtaining seals to these cells was less than 5%. Following the addition of hyaluronidase and neuraminidase to the dissociation procedure, the success rate for obtaining seals increased to greater than 50%. The addition of hyaluronidase and neuraminidase caused no obvious

changes in the electrophysiologic properties of the taste cells.

In the whole cell configuration the taste cells ($N = 75$), at a holding potential of −80 mV, had an input resistance of 1–5 GΩ. At this holding potential the taste cell membrane was highly K^+ selective. Using the standard bath and electrode solutions, the cells had a reversal potential of −80 mV (Nernst potential for K^+ is −90 mV), raising the bath K^+ to 51 mM, by substituting KCl for NaCl in buffer A, shifted the reversal potential to −24 ± 1 mV (mean ± SD; $n = 3$) Nernst potential for K^+ is −25 mV).

In response to depolarizing steps to voltages more depolarized than −20 mV, an outward current was activated, as seen in Fig. 1. The current activated with depolarization and did not inactivate during sustained depolarizations lasting up to 500 msec. Replacing the K^+ in the recording electrode with Cs^+ resulted in complete blockage of this current. Application of TEA (Fig. 2*A*), Ba^{2+} (Fig. 2*C*) or quinine (Fig. 2*B*) to the bath also blocked this current. However, 20 mM 4-aminopyridine, 2 mM phenylthiocarbamide, 0.5 mM denatonium and 1 mM strychnine caused no inhibition. Based on these results the outward current is a delayed rectifier-type K^+ current. The characteristics of this current were unaffected by the antibody-labeling procedure used to identify the taste cells. This was shown by utilizing dissociated cell preparations that were not labeled with antibody. Hyperpolarizing voltage pulses revealed no inward rectifier-type K^+ currents.

A small subset of the taste cells, about 10% (8 of the 75 cells recorded from in the whole cell mode), also had a voltage-dependent, rapidly inactivating, inward current as seen in Fig. 3. The current was activated after depolarization to −40 mV, and its rate of activation proceeded more rapidly with greater depolarization. The current rapidly inactivated within 5 to 10 msec after activation (Fig. 3). Since this current was blocked by 1 μM TTX (Fig. 3*C*), but was not blocked by 0.5 mM amiloride, it is likely that it is due to a voltage-gated Na^+ channel typically found in nerve and muscle cells (Hille, 1984). The taste cells which contained the voltage-dependent Na^+ current also had a voltage-activated tail current seen following repolarization after depolarizing steps to potentials more depolarized than −40 mV (*data not shown*). This tail current was either due to a voltage-dependent chloride current or a nonselective cation current, but was seen too infrequently to permit an analysis of it.

Since amiloride has been shown to inhibit salt and sweet taste responses in humans and animals (Schiffman, Lockhead & Maes, 1983; Heck, Mierson & DeSimone, 1984; Brand, Teeter & Silver, 1985; DeSimone & Ferrell, 1985), a brief search for amiloride-inhibitable currents was made. The cells containing the voltage-dependent Na^+ current do not have any other amiloride-inhibitable currents. Amiloride-inhibitable currents were not found in other taste cells ($n = 9$). The absence of an amiloride-inhibitable current will be discussed later.

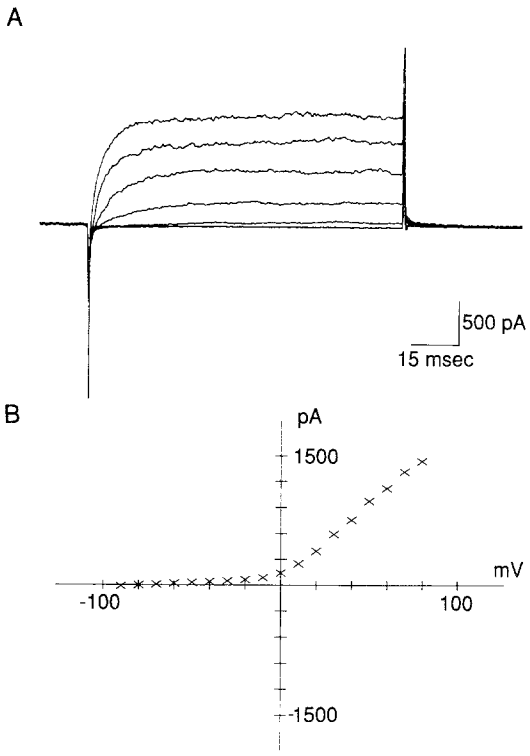

Fig. 1. Whole-cell recording of outward currents from a taste cell. (*A*) Superimposed current responses to 100 msec duration voltage pulses ranging from −20 to +80 mV in steps of 20 mV. Holding potential, −80 mV. Bath, buffer A; pipette, buffer E. (*B*) The steady-state current-voltage relationship for this cell

Since neurosecretory cells frequently contain calcium currents, a search was made for this current by replacing the bath with one containing 130 mM NaCl, 30 mM $BaCl_2$ and utilizing a solution in the pipette where CsCl replaced the KCl, in order to block the K^+ currents. There were no currents observed in these cells ($n = 12$) or in any other taste cells that could be attributed to calcium channels. Calcium channels in many cells are very prone to inactivation during whole-cell recording. The failure to observe calcium currents may be due to our inability to find an appropriate cocktail of agents to preserve the function of the calcium channels.

Nontaste Lingual Epithelial Cells

Recordings from many unlabeled cells ($n > 25$), whether singly or in clumps, showed no active currents. These cells had an essentially linear *I-V* rela-

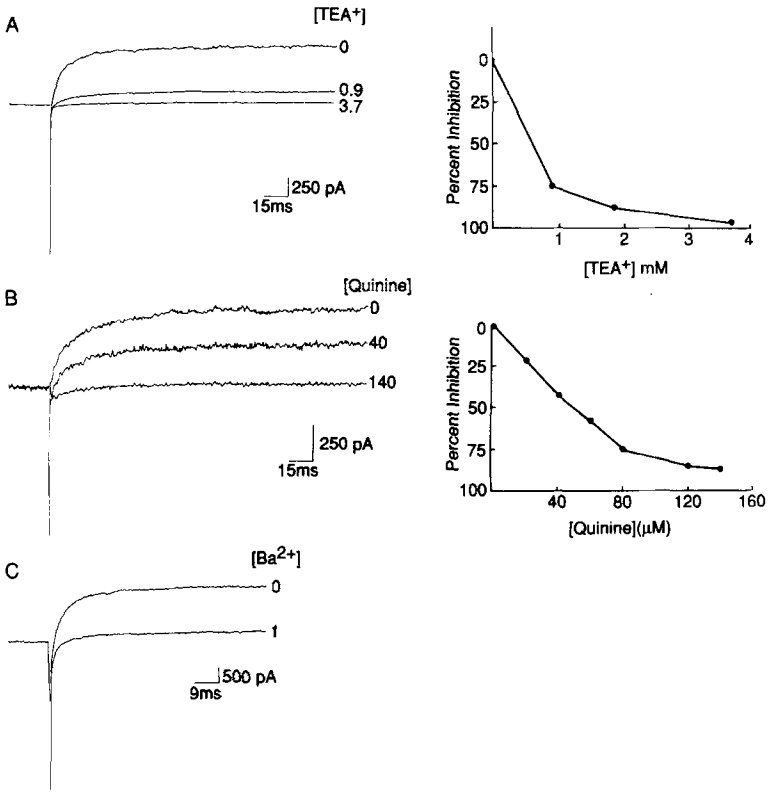

Fig. 2. Inhibition of the outward current. (*A*) Whole-cell recording showing superimposed voltage pulses to +40 mV before addition of TEA, after 0.9 mM TEA and after 3.7 mM TEA. Holding potential, −80 mV. Bath, buffer A; pipette, buffer E. Insert to the right of the current record plots the concentration dependence of the inhibition of the outward current. (*B*) Whole-cell recording showing superimposed voltage pulses to +40 mV before addition of quinine and after the addition of 40 and 140 μM quinine to the bath. Insert to the right shows the concentration dependence of quinine inhibition. All other conditions were as in *A*. (*C*) Inhibition of outward current by 1 mM Ba^{2+}. All conditions were as in *A*.

tionship between −100 and 100 mV with a slope conductance of about 1 nS. Thus, the taste cells have voltage-activated currents and are both antigenically and physiologically different from the remainder of the lingual epithelial cells.

Single Channel Recording

In cell-attached patches a large conductance calcium-dependent K^+ channel was frequently observed. This channel had a mean conductance of 225 pS (±30 pS) (n = 20). The calcium dependence of these channels is illustrated in the inside-out patch shown in Fig. 4, where removal of calcium from the bath by the addition of EGTA dramatically decreased the open probability of the channels. In addition to being calcium dependent the channel was also sensitive to transmembrane voltage, with channel opening in the absence of calcium markedly increased by depolarization as shown in Fig. 4*B*. In cell-attached patches, using electrodes filled with 140 mM KCl, the reversal potential of the single channel current-voltage curve provided an estimate of the resting membrane potential of the taste cells.[1] The measured reversal potential is −70 mV (±5) (n = 23).

A second type of K^+ channel was also observed in excised patches, both inside-out and outside-out. The channel is closed at the resting membrane potential, but the probability of channel opening increased with depolarizations to voltages more positive than −20 mV (Fig. 5*A*). It has a linear single channel current-voltage relationship with a single channel conductance of 90 pS (Fig. 5*B*). In addition, the channels were blocked by 5 mM TEA^+. These properties are similar to many delayed rectifier potassium channels (Hille, 1984). By averaging the current response due to repetitive pulses to a given voltage, for an outside-out patch containing several 90 pS channels, an *I-V* curve was generated that superimposes on a whole-cell *I-V* curve for voltages less depolarized than +40 mV (*compare* Figs. 1*B* and 5*C*). This suggests that the voltage-dependent activation seen in the whole-cell recordings is due to activation of the 90-pS channels below +40 mV. At voltages more depolarized than +40 mV the contribution of the K(Ca) channels probably accounts for the deviation from the whole-cell current-voltage curve.

In outside-out patches only the 90-pS, voltage-dependent channel is seen, probably due to the inclusion of 10 mM EGTA in the patch pipette. Attempts to use lower EGTA concentrations were unsuccessful due to rapid clogging of the electrode tip after attaining the whole-cell configuration.

[1] This assumes that the intracellular potassium concentration is approximately 140 mM and therefore the Nernst potential for potassium is small. In fact, it is unlikely that the intracellular potassium concentration is as high as 140 mM, so the reversal potential will underestimate the actual membrane potential, which is likely to be even more negative.

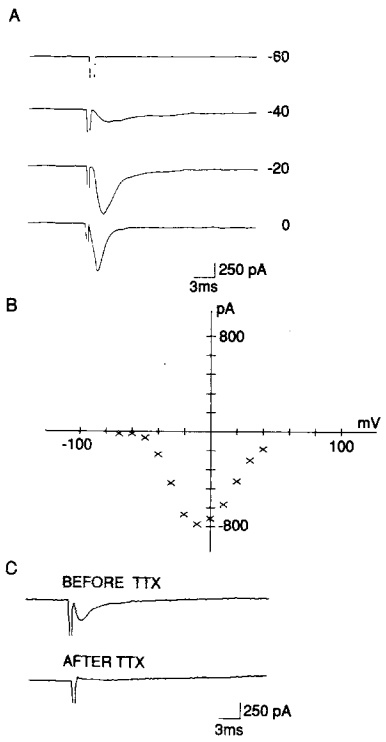

Fig. 3. Whole-cell recording of a taste cell containing the rapidly inactivating inward current. (*A*) Currents were obtained by voltage steps to the potential listed to the right of each trace from a holding potential of −80 mV. The pipette contained 140 mM CsCl to block the outward current; bath, buffer A. The initial capacitive transient has been truncated in the figure. (*B*) Corresponding peak inward current-voltage relationship. (*C*) Inhibition of the rapidly inactivating inward current by 1 μM TTX. Top trace shows current response to a voltage pulse to −10 mV before application of TTX; lower trace shows the response of the same cell to a similar voltage pulse after application of TTX to the bath. Holding potential, −80 mV. Bath, buffer A; pipette, buffer E

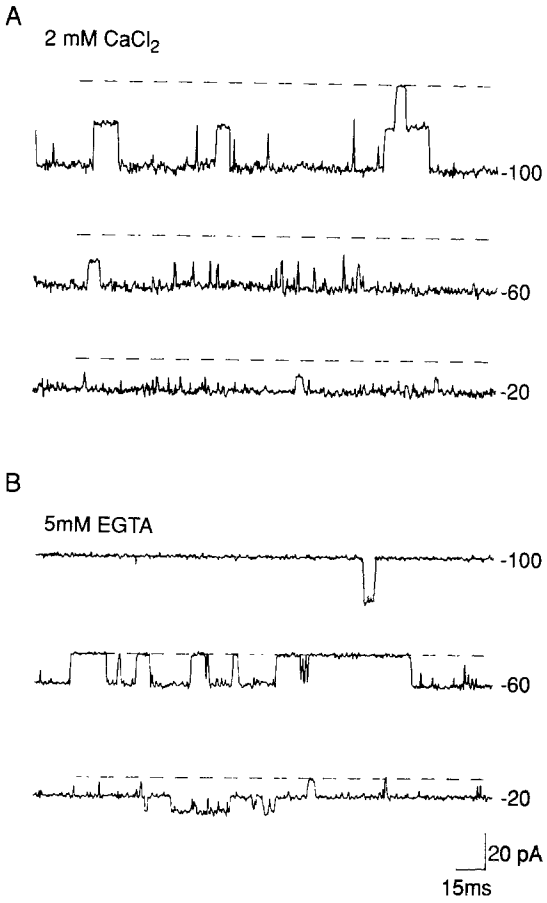

Fig. 4. Inside-out patch recording illustrating the voltage and calcium dependence of the large conductance potassium channel. (*A*) Single channel recording at three holding potentials, listed to the right of each trace, in the presence of 2 mM Ca^{2+} in the bath. Channels open downward. (*B*) Same patch following the addition of 5 mM EGTA to the bath. Note the marked decrease in channel opening events

Discussion

Until recently, studies of the mechanism of sensory transduction in the mammalian gustatory system were limited by the inability to study the primary sensory transducing cells in the taste buds. We have now developed a preparation of dissociated rat lingual epithelium that permits the direct study of the taste cells (Akabas et al., 1988). In this report we have described the electrophysiologic properties of rat taste cells.

The resting membrane input resistance of the rat taste cells was found to be greater than 1 GΩ. The resting membrane is highly selective for potassium, and this correlates with the measured resting membrane potential of −70 mV. The impedance and resting potential reported here are significantly greater than those reported in the literature that were obtained in situ with intracellular microelectrodes (Sato, 1980). The lower values reported for the in situ measurements probably reflect leakage around the intracellular recording electrode due to damage to the cell membrane during insertion of the electrode. The leak conductance is large enough so that a significant portion of the changes in membrane potential following exposure to sapid solutions might be due to passage of ions through the leak rather than through the cell membrane, making it difficult to interpret the observed changes.

On the basis of their electrophysiological characteristics, there are two populations of cells within the rat taste bud, one containing only a K^+ current and the other containing both a K^+ current and a voltage-dependent Na^+ current. This represents a significant difference from the amphibian taste buds where all of the cells in the taste buds appear to have the same basic set of ion channels (Avenet & Lindemann, 1987; Kinnamon & Roper, 1988).

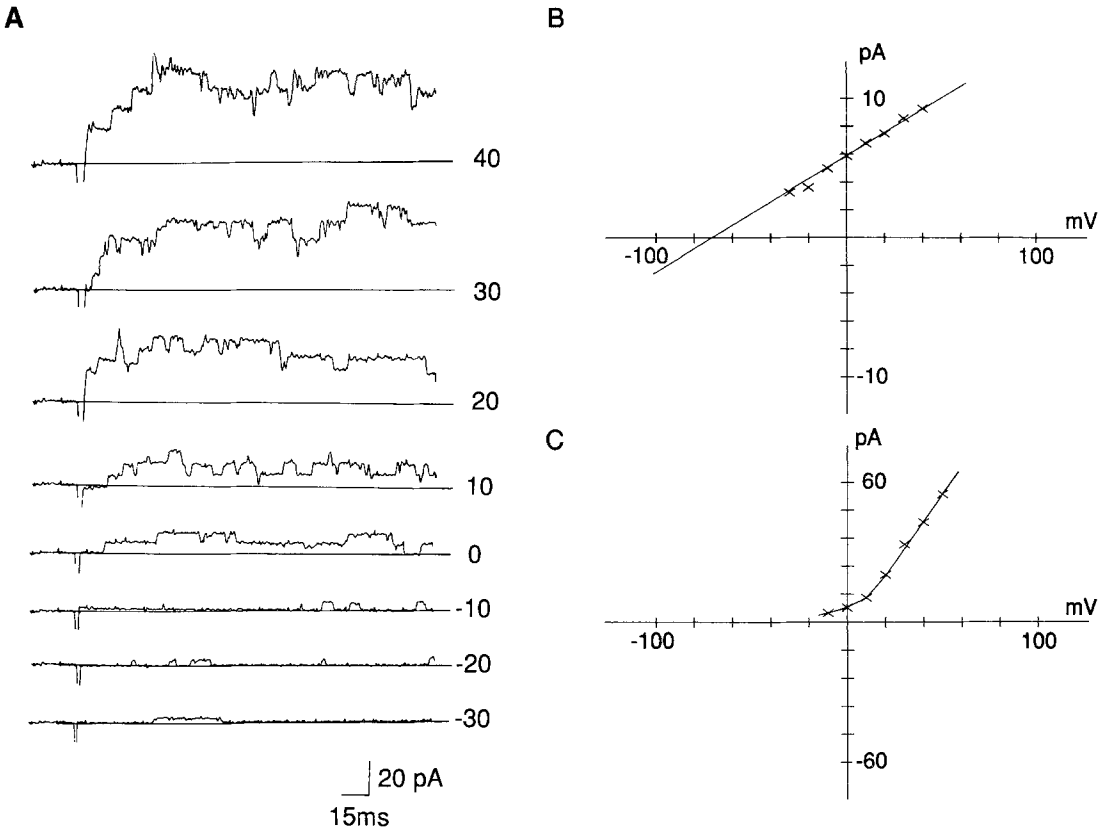

Fig. 5. Outside-out patch recording illustrating the 90-pS potassium channel. (*A*) Response of a patch containing six channels to depolarizing voltage pulses to the potentials listed to the right of each trace. Note the increase in the number of open channels with increasing depolarization. Holding potential, −80 mV. Bath, buffer A; pipette, buffer E. (*B*) Single channel current-voltage plot for the patch shown in *A*. Potassium reversal potential calculated from Nernst equation is −78 mV. (*C*) Corresponding steady-state current-voltage relationship for the patch in *A* obtained by summing the current response of five pulses to each given voltage

There are several possible explanations for the existence of subpopulations of cells within the taste buds. For example, they may represent functionally distinct subgroups, such as supporting cells and sensory cells or sensory cells involved in the transduction of distinct taste modalities. Morphological studies have identified several subpopulations of cells within the taste buds based mainly on differences in the intensity of staining at both the light and electron microscopic level (Farbman, 1965; Murray et al., 1969; Kinnamon et al., 1985). The origin of these differences and whether they have functional significance or are related to changes in the avidity for stain as the cells age is unclear. Alternatively, the observed differences in cell properties may reflect the state of functional differentiation of taste cells. The taste cells in the rat undergo a 13-day life cycle from basal cells to cell death (Biedler & Smallman, 1965; Delay, Kinnamon & Roper, 1986). As they differentiate they may express different ion channels, as has been described in other systems (Spitzer, 1979). At present it is not possible to distinguish between these alternatives. The scarcity of Na^+ channel containing cells has precluded extensive characterization. Further studies will be needed to clarify these issues.

Some bitter substances, such as quinine and TEA, are also potent K^+ channel blockers. This has led to the suggestion that one mechanism of bitter taste transduction involves K^+ channel blocking (Ozeki, 1971). However, the results of this study suggest that bitter taste is probably independent of the ability to block K^+ channels. External Ba^{2+} is a potent blocker of the K^+ current, but does not taste bitter at 5 mM (*personal observation*). In addition, other bitter substances, such as strychnine, denatonium and phenylthiocarbamide do not block the K^+ current in the rat taste cells. Our own studies of the process of bitter taste transduction suggest that it is mediated by a cell surface receptor, which upon binding the appropriate ligand stimulates the synthesis of an intracellular second messenger, result-

ing in the release of calcium from internal stores (Akabas et al., 1988). An increasing amount of evidence is accumulating that intracellular second messengers are important in the transduction of various taste modalities (Nagahama, Kobatake & Kurihara, 1982; Avenet et al., 1988; Hwang et al., 1989; Striem et al., 1989).

Because the taste cells are epithelial cells, the localization of the channels in the apical or basolateral domain may serve an important role in the transduction process. In mudpuppy, a class of K^+ channels has been found to be localized selectively in the apical domain, whereas the Na^+, Ca^{2+} and other K^+ channels are either unlocalized or only in the basolateral domain (Kinnamon, Dionne & Beam, 1988; Roper & McBride, 1989). The importance of vectorial current flow through the taste cells in the transduction of certain taste modalities is suggested by the observation of changes in the transepithelial short-circuit current in isolated lingual epithelium in response to perfusion of sapid substances on the apical surface (Heck et al., 1984; Mierson et al., 1985; Simon, Robb & Schiffman, 1988). In particular, the lingual epithelium from a variety of mammalian species has an amiloride-inhibitable Na^+ current (Heck et al., 1984; Simon et al., 1988). In other experiments, micromolar amiloride applied topically to the tongue has been shown to diminish the perceived intensity of NaCl as being salty in humans (Schiffman et al., 1983) and to decrease or block the response of the chorda tympani nerve to lingual stimulation with NaCl in rats and hamsters (Brand et al., 1985; DeSimone & Ferrell, 1985). This suggests that an amiloride-sensitive Na^+ transport pathway is involved in salt taste transduction. An amiloride-sensitive current has been found in frog taste cells (Avenet & Lindemann, 1988). The failure to find an amiloride-sensitive current in the rat taste cells may be due to several factors. The channels may be expressed in only a small subset of the taste cells, those responsive to salty stimuli. Single axon recordings from the glossopharyngeal nerve, which innervates the circumvallate papillae, suggest that only about one quarter of the sensory axons are responsive to salty solutions (Frank, 1975). However, this does not imply that 25% of the taste bud cells are salt sensitive cells because many of the cells within a taste bud may not be sensory cells. At present there is no way to distinguish sensory from nonsensory cells within the taste bud. In our limited survey we may not have sampled a salt-sensitive taste cell. It should also be noted that trypsin damages the amiloride-sensitive Na^+ channel in some tissues (Garty & Edelman, 1983). Thus, the dissociation procedure used in our experiments may have destroyed the channels prior to our search for them.

One of the central questions in gustatory transduction is: what is the mechanism of specificity for various taste modalities? In mudpuppy all of the taste cells have the same complement of ion channels and no subpopulations have been observed (Kinnamon & Roper, 1988). If all of the taste cells are identical in terms of their electrophysiological properties it is unclear how the animal could use these channels to discriminate between different substances, a crucial function of the gustatory system. This raises the question of whether the initial step in the transduction of tastes other than salt involve cell surface receptors specific for various classes of sapid substances. Our studies of bitter taste strongly suggest that cell surface receptors are important (Akabas et al., 1988). Extensive studies in catfish have demonstrated specific receptors for alanine and arginine which are segregated into separate populations of receptor cells (Cagan & Boyle, 1984; Caprio & Byrd, 1984; Brand et al., 1987). Similarly, studies of sweet taste strongly implicate the existence of cell surface receptors for sweet molecules (Schiffman, Hopfinger & Mazur 1986; Jakinovich & Sugarman, 1988).

Further study of these cells and the identification of specific markers for subpopulations of the sensory cells will facilitate clarification of the relationship between the electrophysiologic properties of the taste cells and the process of transduction of specific taste modalities.

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