Mouse Taste Cells With Glialike Membrane Properties

ALBERTINO BIGIANI
Dipartimento di Scienze Biomediche, Sezione di Fisiologia, Università di Modena e Reggio Emilia, 41100 Modena, Italy

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Bigiani, Albertino. Mouse taste cells with glialike membrane properties. J Neurophysiol 85: 1552–1560, 2001. Taste buds are sensory structures made up by tightly packed, specialized epithelial cells called taste cells. Taste cells are functionally heterogeneous, and a large proportion of them fire action potentials during chemotransduction. In view of the narrow intercellular spaces within the taste bud, it is expected that the ion composition of the extracellular fluid surrounding taste cells may be altered significantly by activity. This consideration has led to postulate the existence of glialike cells that could control the microenvironment in taste buds. However, the functional identification of such cells has been so far elusive. By using the patch-clamp technique in voltage-clamp conditions, I identified a new type of cells in the taste buds of the mouse vallate papilla. These cells represented about 30% of cells patched in taste buds and were characterized by a large leakage current. Accordingly, I named them “Leaky” cells. The leakage current was carried by K+, and was blocked by Ba2+ but not by tetraethylammonium (TEA). Other taste cells, such as those possessing voltage-gated Na+ currents and thought to be chemosensory in function, did not express any sizeable leakage current. Consistent with the presence of a leakage conductance, Leaky cells had a low input resistance (~0.25 GΩ). In addition, their zero-current (“resting”) potential was close to the equilibrium potential for potassium ions. The electrophysiological analysis of the membrane currents remaining after pharmacological block by Ba2+ revealed that Leaky cells also possessed a Cl– conductance. However, in resting conditions the membrane of these cells was about 60 times more permeable to K+ than to Cl–. The resting potassium conductance in Leaky cells could be involved in dissipating rapidly the increase in extracellular K+ during action potential discharge in chemosensory cells. Thus Leaky cells might represent glialike elements in taste buds. These findings support a model in which specific cells control the chemical composition of intercellular fluid in taste buds.

INTRODUCTION

Taste transduction relies on several molecular and cellular processes (reviewed in Herness and Gilbertson 1999; Lindemann 1996). Among them, firing of action potentials by taste cells appears to be an important step in the processing of the chemosensory information at the peripheral level. Action potentials in taste cells are generated by the same ionic mechanisms described for other excitable cells, such as neurons. Namely, they derived from the interplay of voltage-gated ionic conductances that caused ionic fluxes across the plasma membrane (Chen et al. 1996; Kinnamon and Roper 1987; Roper 1983). Given the narrow intercellular spaces between cells inside taste buds (Murray 1973), it is expected that ion concentrations might change during action potential discharges. In particular, potassium ions are extruded from taste cells for membrane repolarization and can accumulate in the extracellular space. Increased extracellular K+ concentration, [K+], can disrupt membrane excitability. A similar situation is observed also in other excitable tissues, such as in CNS and in the retina, where active cells determine variations of the extracellular ion concentrations, most notably K+ concentration (reviewed in Somjen 1979; Sykova et al. 1998; Walz 1989). In these tissues, glia cells possess membrane properties, such as a conspicuous “resting” K+ conductance, that enable them to distribute rapidly extracellular K+ from active to resting areas of the tissue (“spatial buffering”), thus avoiding dangerous accumulation of this cation in the extracellular space (Sykova et al. 1998; Walz 1989). It has been suggested that also in taste buds a glialike control of the extracellular ion concentrations should be performed by some not-yet identified cells (Lindemann 1996). Although several patch-clamp studies have been carried out on single taste cells, up to now there are no functional data on such cells. In this paper I have addressed the issue of functional glialike cells in taste buds by studying the membrane properties of taste cells in the mouse vallate papilla with the patch-clamp recording technique.

METHODS

Tissue preparation

Adult male C57BL/6J mice were used in this study. Vallate taste buds were isolated with an enzymatic-mechanical procedure (e.g., Béhé et al. 1990; Gilbertson et al. 1993; Miyamoto et al. 1996). Briefly, mice were deeply anesthetized by CO2, followed by dislocation of cervical vertebrae. Tongues were rapidly removed and placed in Tyrode solution (mM: 120 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, 10 HEPES, 10 glucose, 10 Na pyruvate, and 20 Na methanesulfonate, pH 7.4 with NaOH). Two milligrams of elastase (Worthington Biochemical, Freehold, NJ), and 2 mg of dispase (grade II; Boehringer Mannheim, Mannheim, Germany) in 1.0 ml of Tyrode solution were injected (0.2–0.4 ml/tongue) between the lingual epithelium and muscle layer. Tongues were incubated in Ca2+-free Tyrode solution at 30°C for ~15–20 min. After incubation, the lingual epithelium could be peeled free from the underlying tissue with gentle dissection. The freed epithelium was pinned serosal side up in a silicone elastomer (Sylgard)—lined Petri dish and incubated in Ca2+-free Tyrode solution for ~5–10 min to loosen the attachments of taste buds to the papilla. Vallate taste buds were removed from the epithelium by gentle suction with a fire-polished pipette (tip diameter, about 50 μm) and plated on the bottom of a chamber that consisted of a standard glass...
Electrophysiological identification of taste cells

I studied the membrane currents elicited in mouse taste cells from the vallate papilla by depolarizing the membrane under whole cell voltage-clamp. I chose a holding potential of −84 mV (LJP corrected) as standard reference potential so that membrane currents from different taste cells could be compared. In these conditions, I patched a total of 112 cells and was able to identify three groups of cells according to their membrane currents.

About 53% of recorded cells (59 of 112 cells) displayed voltage-dependent inward and outward currents (Fig. 1A).

**FIG. 1.** Membrane currents (I_m) characterizing 3 different populations of taste cells in mouse vallate taste buds. Cells were held at −84 mV and stepped in 10-mV increments from −74 to +16 mV. A: “Na/OUT” cells were characterized by voltage-dependent inward currents carried by Na⁺, and outward currents carried by K⁺ and/or Cl⁻. Outward currents typically activated at about −30 mV, −20 mV, as shown by the current-voltage (I-V) relationship on the right. B: “OUT” cells possessed only voltage-dependent outward currents, which were carried by K⁺ and/or Cl⁻. Outward currents activated at a voltage range similar to the one observed for Na/OUT cells. C: “Leaky” cells were characterized by the presence of conspicuous leakage currents that were almost negligible in the other 2 groups of cells (compared the I-V plots for the voltage range between −84 and −20 mV). In all I-V plots, outward currents (I_out) were measured at the end of the 30-ms voltage pulses. Each point represents the means ± SE from 35 Na/OUT cells, 13 OUT cells, and 31 Leaky cells. V_m: membrane potential.
Ionic substitution and pharmacological dissection showed that the inward current was mediated by sodium ions, whereas the outward current was mediated by potassium and/or chloride ions (data not shown). The relative proportion of potassium current and chloride current to the outward currents was highly variable. Thus for convenience I named this type of cells as “Na/OUT” cell, where “Na” stands for the presence of sodium currents and “OUT” for the presence of outward (K\textsuperscript{+}/Cl\textsuperscript{−}) currents.

Another group of taste cells (about 15%; 17 of 112 cells) displayed only voltage-dependent outward currents similar to those found in the previous group (Fig. 1B). For convenience, I named this second type of cells as “OUT” cell to indicate that they possessed only outward (K\textsuperscript{+}/Cl\textsuperscript{−}) currents.

Taste cells similar to the Na/OUT and OUT types have been already described in mouse (Furue and Yoshii 1997; Spielman et al. 1989) as well as in other vertebrates (Akabas et al. 1990; Avenet and Lindemann 1987; Béhé et al. 1990; Bigiani and Roper 1993; Chen et al. 1996; Miyamoto et al. 1988; Sugimoto and Teeter 1990). These cells, or at least of part of them, may represent sensory cells in different stages of their normal turnover (e.g., Mackay-Sim et al. 1996).

In addition, I identified a new cell type (36 of 112 cells) characterized by the presence of a strong leakage current (Fig. 1C). For this reason, I named it as “Leaky” cell. The leakage component of the whole cell current could be readily appreciated from the I-V plot (Fig. 1, right). In the voltage range between approximately −85 and −20 mV, Leaky cells displayed a higher membrane conductance (slope of the I-V curve) than the other two groups of cells. Leaky cells were further distinguished by the high negative value of their zero-current potential (V\textsubscript{0}, a rough estimation of the cell’s resting potential in whole cell recordings) (Bigiani et al. 1996), −74 ± 0.7 mV (mean ± SE, n = 29) compared with that displayed by the other two groups of cells: −47 ± 2.7 mV (Na/OUT cells; n = 35), −36 ± 4.4 mV (OUT cells; n = 12). Consistent with the presence of the leakage current, Leaky cells also displayed a low input resistance (R\textsubscript{in}, Fig. 2). Finally, Leaky cells had a larger surface area than Na/OUT cells and OUT cells, as indicated by measurements of the cell membrane capacitance (C\textsubscript{m}; Fig. 2).

Membrane currents in Leaky cells

A more complete I-V characteristic for Leaky cell’s membrane was obtained by applying hyperpolarizing and depolarizing voltage pulses from a holding potential of −84 mV. Representative recordings from two Leaky cells are shown in Fig. 3A. Leakage currents were apparent in all the voltage range tested. In some cells, voltage-dependent outward currents were also present (Fig. 3A, bottom records). Figure 3B shows the I-V plot of the mean values of membrane currents recorded from 27 Leaky cells. For membrane voltages greater than about −20 mV, the I-V relationship was nonlinear, suggesting the presence of a voltage-dependent component in the membrane current in addition to the leakage component. This

![Image](http://jn.physiology.org/)

**FIG. 2.** Zero-current potential (V\textsubscript{0}), input resistance (R\textsubscript{in}), and membrane capacitance (C\textsubscript{m}) of cell populations identified in mouse vallate taste buds. Leaky cells differed significantly (P < 0.05) from the other 2 cell types for all the reported parameters. Data from 35 Na/OUT cells, 12 OUT cells, and 29 Leaky cells.

![Image](http://jn.physiology.org/)

**FIG. 3.** Membrane currents (I\textsubscript{m}) in Leaky cells of the vallate taste buds. A: representative current records from 2 cells held at −84 mV and stepped from −124 to +26 mV, in 10-mV increments. In addition to the leakage currents, voltage-dependent outward currents were also detectable, although their amplitude varied considerably form cell to cell. B: current-voltage (I-V) relationships of the membrane currents recorded from 27 Leaky cells. In the voltage range between −124 mV and about −20 mV, the I-V relationship becomes nonlinear. I\textsubscript{m}, membrane current measured at the end of 30-ms voltage pulses; V\textsubscript{m}, membrane potential.
outward rectification varied considerably among Leaky cells (compare records in Fig. 3A).

The membrane conductance responsible for the leakage current in Leaky cells was selective for potassium ions: elevation of external potassium concentration resulted in a marked increase in current amplitudes associated with a positive shift of the I-V curve (Fig. 4). Current reversal potentials were close to the theoretical equilibrium potentials (E_K) for each of the imposed ionic gradients for K^+, indicating that currents were almost exclusively carried by K^+ ions. The mean membrane potentials were −75 ± 2 mV (n = 8), −60 ± 1 mV (n = 4), −47 ± 1 mV (n = 7), and −26 ± 1 mV (n = 6) in 5, 10, 20, and 50 mM K^+-containing solutions, respectively. These shifts in membrane potential suggest an approximately −52-mV change in potential per 10-fold change in [K^+]_o, supporting the relative selectivity of the leakage conductance for K^+ ions. This value is similar to that previously observed for currents through the inwardly rectifying K^+ channels in astrocytes (Ransom and Sontheimer 1995), ventricular cells (Sakmann and Trube 1984), and rat taste cells (Sun and Herness 1996).

To establish the pharmacological properties of the leakage currents, I used known blockers of K^+ channels such as tetraethylammonium (TEA) and barium (reviewed in Castle et al. 1989; Rudy 1988). Figure 5 illustrates data that are representative of all Leaky cells that were tested with TEA (n = 5). TEA (20 mM) was unable to affect the linear (leakage) component of the whole cell currents. However, TEA did block the voltage-dependent component of the outward currents (Fig. 5). On the contrary, the leakage current was sensitive to Ba^2+ in all cells tested (n = 14; Fig. 6). This result supported the view that the leakage current was K^+ dependent and indicated that it exhibited a specific sensitivity to Ba^2+, similarly to the inward rectifying K^+ currents (I_{IR}) described in glia cells (e.g., Linn et al. 1998; Newman 1989; Ransom and Sontheimer 1995). It is interesting to note that in the presence of Ba^2+, the reversal potential in the I-V plot shifted toward zero (Fig. 6B), whereas no change could be detected when TEA was applied (Fig. 5B).

Steady inward currents and resting conductances in Leaky cells

Leaky cells held at approximately −80 mV, that is, below their zero-current potential, showed a steady inward current (Fig. 7A). Ba^2+ caused an increase in this holding current recorded when the cell was kept at approximately −80 mV (Fig. 7A). Concomitant with the increase in holding current, an increase in the membrane resistance could be detected, as indicated by measurement of cell input resistance (R_{in}; Fig. 7A). The average increase in R_{in} was approximately sixfold (Fig. 7B; P < 0.001). The change in R_{in} was consistent with channels being closed by Ba^2+.

Ba^2+ strongly affected also the membrane potential of Leaky cells: 10 mM Ba^2+ caused about 50 mV depolarization (Fig. 8A; P < 0.001). This shift in membrane potential suggested that Ba^2+ blocked a membrane conductance directly involved in maintaining the resting membrane potential. As indicated by the concentration-inhibition curve shown in Fig. 8B, Ba^2+ induced half-maximal reduction of V_0 at a concentration of ~0.6 mM. This result was consistent with the sen-
sitivity of the K⁺ leakage current to Ba²⁺ described above (see Fig. 6).

The increase in the steady inward current in the presence of Ba²⁺ could be due to unmasking of an outward flux of Cl⁻ or of an inward flux of Na⁺, which were the main ions, in addition to K⁺, in my experimental conditions. At a membrane potential of −84 mV and in regular Tyrode, both ions were not at the Nernst equilibrium, as indicated by their equilibrium potentials (E₇_Cl ≈ −1 mV; E₇_Na ≈ +130 mV). The involvement of Cl⁻ conductance in the steady inward current was tested by apply-

**FIG. 6.** Sensitivity of membrane current (Iₘ) to Ba²⁺ in Leaky cells. A: a Leaky cell was held at −84 mV and stepped in 10-mV increments from −124 to +26 mV. Ba²⁺ (10 mM) blocked the linear component (leakage current) of the whole cell currents. B: I-V relationships for the current records shown in A. Note that the reversal potential (arrows) in the I-V plot shifted toward zero when the leakage current was blocked by Ba²⁺. Membrane current was measured at the end of 30-ms voltage pulses. Vₘ, membrane potential.

**FIG. 7.** Effect of Ba²⁺ on input resistance (Rᵢ) of Leaky cells. A: change in Rᵢ of a Leaky cell during application of 10 mM Ba²⁺. Rᵢ was monitored by application of −10-mV steps from a holding potential of approximately −80 mV. Input resistance increased from 0.5 to 3.1 GΩ during the response to barium, indicating that membrane conductance was reduced. Iₘ, membrane current. B: Rᵢ in 4 Leaky cells measured in regular Tyrode (control) and during application of 10 mM Ba²⁺. The difference was statistically significant (P < 0.001).

**FIG. 8.** Effect of Ba²⁺ on zero-current potential (V₀) of Leaky cells. A: V₀ in 9 Leaky cells measured in regular Tyrode (control) and during application of 10 mM Ba²⁺. V₀ was reduced more than 3-fold in the presence of Ba²⁺. The difference was statistically significant (P < 0.001). B: concentration-inhibition curve for the effect of Ba²⁺ on V₀. Points represent means ± SE from 7 to 13 measurements. Data points were fitted using the logistic equation and gave an IC₅₀ ≈ 0.6 mM, and a Hill coefficient ≈ 0.8.

**FIG. 9.** Membrane currents (Iₘ) in Leaky cells during block of the leakage currents by Ba²⁺. A: application of 10 mM Ba²⁺ induced an increase (downward deflection) of the steady inward current in a Leaky cell held at approximately −80 mV. Co-application of 500 μM 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), a known Cl⁻ conductance blocker in taste cells (e.g., Herness and Sun 1999; Taylor and Roper 1994). Figure 9A shows the steady inward current recorded from a Leaky cell during bath application of 10 mM Ba²⁺. In this condition, 500 μM DIDS markedly reduced the inward currents (Fig. 9A), indicating that
V_{m} (nV)

\[ P_{Cl}/P_{K} = 0.016 \]

\[ r^2 = 0.998 \]

FIG. 10. Effect of variations in [K\(^+\)]\(_o\) on the value of resting membrane potential (V_{0}) in Leaky cells. Resting membrane potential was calculated by correcting V_{0} for the shunt to ground by seal resistance (see METHODS). Points represent means ± SE from 4 to 8 measurements. Data points were fitted using the Goldman-Hodgkin-Katz equation in the hypothesis that the membrane was permeable only to K\(^+\) and to Cl\(^-\). The best fit of the data points was obtained for a permeability ratio \( P_{Cl}/P_{K} \) of 0.016.

it was mediated by a Cl\(^-\) flux. The mean percent reduction in the inward current induced by DIDS was 56 ± 12\% \( (n = 4) \).

The involvement of a Na\(^+\) conductance in setting up the steady inward current in the presence of Ba\(^{2+}\) was tested by replacing Na\(^+\) with N-methyl-D-glucamine or choline in the bath. In these conditions, an upward deflection in the current trace should be expected if a Na\(^+\) conductance was operating in the cell. However, as shown in Fig. 9B, replacement of extracellular Na\(^+\) did not cause any detectable upward deflection in the current trace \( (n = 3) \).

In summary, the data are consistent with the steady inward current in the presence of Ba\(^{2+}\) being mediated by a Cl\(^-\) conductance. The contribution of this conductance in the generation of the resting potential in Leaky cells seems to be, however, smaller compared with the K\(^+\) conductance. V_{0} in Leaky cells was close to the potassium equilibrium potential (about −88 mV) both when the pipette solution contained KCl (V_{0} = −74 ± 0.7 mV; \( n = 29 \)) or K gluconate (−75 ± 0.8 mV; \( n = 7 \)). To estimate the relative membrane permeabilities of Leaky cells to K\(^+\) and Cl\(^-\) in resting conditions, V_{0} was measured at different [K\(^+\)]\(_o\) and then corrected for the shunt to ground by the seal resistance (see METHODS). This correction allowed to evaluate the resting potential (V_{r}) in Leaky cells. Figure 10 shows that V_{r} values at different [K\(^+\)]\(_o\) could be well fitted by the Goldman-Hodgkin-Katz equation for a permeability ratio \( P_{Cl}/P_{K} \) of about 0.016. This finding indicated that in resting conditions the membrane of Leaky cells was, on average, about 60 times more permeable to K\(^+\) than to Cl\(^-\).

Voltage-dependent conductances in Leaky cells

In addition to the leakage K\(^+\) conductance, some Leaky cells also possessed voltage-dependent currents. In Fig. 3A, bottom traces, for example, an early, downward deflection can be noted in a few records, suggesting that the depolarizing steps activated a voltage-dependent inward current. Moreover, the outward rectification described above (see, for example, Fig. 3A, and the currents in Fig. 6A during Bu\(^{2+}\) treatment) was suggestive of the occurrence of voltage-dependent outward current. A direct demonstration of the presence of voltage-dependent currents was obtained by subtracting the leakage component from the whole cell currents. Figure 11 shows representative recordings from two Leaky cells in control conditions (left traces) and after subtracting electronically leakage currents (middle traces). In one cell (Fig. 11A), leak subtraction did not unmask any voltage-dependent currents (Fig. 11A, records in the middle, and corresponding I-V plot on the right). On the contrary, in the other cell (Fig. 11B), leak subtraction revealed the presence of two types of voltage-dependent currents, a transient inward current (Fig. 11B, ●) and a sustained outward current (Fig. 11B, ○). The corresponding I-V curves are reported in Fig. 11B on the right. The inward current was blocked by 1 \( \mu \)M TTX \( (n = 3) \); data not shown), indicating that it was a Na\(^+\) current, whereas the outward current was blocked by 20 mM TEA \( (n = 5) \); data not shown), indicating that it was a K\(^+\) current. The occurrence of these voltage-dependent currents as well as their magnitude varied considerable among Leaky cells. After leak subtraction, voltage-dependent Na\(^+\) currents \( (I_{Na}) \) were detected in about 45\% of tested cells \( (10 \) of 22 cells), with an average peak value of −106 ± 29 pA \( (range \ -22 to −311 \) pA; \( n = 10 \)). In the same conditions, voltage-dependent K\(^+\) currents \( (I_{K}) \) were present in about 86\% of tested cells \( (19 \) of 22 cells). \( I_{K} \) amplitude, evaluated at the end of a 30-s depolarizing step to +46 mV, averaged 258 ± 38 pA \( (range \ 60–3,035 \) pA; \( n = 44 \)), whereas voltage-dependent outward currents (mediated by K\(^+\) and/or

![FIG. 11. Membrane currents in Leaky cells after leak subtraction to reveal voltage-dependent currents. A: A Leaky cell was held at −84 mV and stepped in 10-mV increments from −74 to +26 mV. Membrane currents \( (I_{m}) \) were due mainly to the leakage conductance, as indicated by the currents records obtained after leak subtraction. B: in another Leaky cell, the same experimental protocol used for the cell in A elicited both leakage currents and voltage-dependent currents, as indicated by the records obtained after leak subtraction and by their corresponding I-V relationship. Pharmacological dissection revealed that the transient inward currents (●) were Na\(^+\) currents, whereas the sustained outward currents (○) were K\(^+\) currents.](http://jn.physiology.org/doi/pdf/10.220.33.1)
in my knowledge have reported the findings described in this study. This is surprising, and at the moment I am unable to explain this difference. A possibility is that patched cells with low input resistance may have been rejected in those studies. In patch-clamp recordings, poor seal resistances are associated with “leakage” current shunted across the leakage conductance around the pipette-membrane seal. In Leaky cells of the mouse taste buds, the highly negative values of $V_o$ and the sensitivity of the leakage current to barium were inconsistent with damaged cells.

Glial cells in the nervous system as well as in sensory organs, such as the retina, are endowed with several kinds of potassium conductances. Among them, the inwardly rectifying potassium (KIR) current is thought to be involved in the control of [K$^+$]$_o$ (Abbott 1998; Barres et al. 1990; Walz 1989). As the name indicates, typical KIR conductance exhibits strong rectification, with little current conducted outwardly. However, several types of this conductance are known, and some of them display weak or no rectification (reviewed in Nichols and Lopatin 1997). It is then tempting to speculate that the resting K$^+$ conductance in Leaky cells may be a KIR-like conductance that exhibits weak or no rectification. Further characterization of the leakage current is required to establish whether it derived from the activity of a single type of channel or not.

In taste cells possessing voltage-gated TTX-sensitive Na$^+$ channels (that is, Na/OUT cells in my nomenclature), appropriate ionic manipulations have allowed to document the presence of the KIR conductance (Kinnamon and Roper 1988; Sun and Herness 1996). These cells, however, present low membrane conductance in normal ionic conditions, suggesting that the magnitude of KIR conductance is low unless enhanced with appropriate experimental maneuvers. Leaky cells, on the contrary, possess a very large membrane conductance in resting conditions (Figs. 1 and 2). In addition, KIR conductance in Na/OUT taste cells presents a strong inward rectification and a partial sensitivity to 20 mM TEA (Kinnamon and Roper 1988; Sun and Herness 1996). Thus it is conceivable that the K$^+$ conductance expressed by Leaky cells is quite different from the KIR described in other taste cells. A similar situation has been described in the goldfish sacculus, where supporting cells have a unique inwardly rectifying K$^+$ conductance, characterized by weak inward rectification and insensitivity to TEA, which is different from the typical inwardly rectifying current of hair cells (Sugihara and Furukawa 1996). It has been suggested that this unique KIR conductance in supporting cells of the inner ear likely give them a certain K$^+$-buffering function in both influx and efflux (Sugihara and Furukawa 1996).

In addition to leakage K$^+$ currents, some Leaky cells possessed voltage-dependent Na$^+$ currents and K$^+$ currents. The physiological significance of these currents in Leaky cells remains to be fully elucidated. It is unlikely that these currents are involved in the generation of action potentials: Leaky cells, by virtue of their large resting K$^+$ conductance, exhibit a very low membrane input resistance that would shortcut any depolarizing currents such as those associated with the activation of voltage-dependent Na$^+$ channels. It is interesting to note that glial cells, such as astrocytes and Schwann cells, are endowed with a vast array of voltage-dependent ion channels, including Na$^+$ channels (for re-
view, see Barres et al. 1990; Ritchie 1992; Sontheimer et al. 1996). However, they are unable to fire action potentials, at least in physiological conditions (Sontheimer et al. 1996).

Vertebrate taste cells are structurally heterogeneous, and different cell morphotypes have been identified, such as elongated cells (subdivided in type I, type II, and type III cells), and round basal cells (reviewed in Lindemann 1996; Roper 1989). At the moment, I do not have data on the structural features of Leaky cells. However, in a few cases (n = 4), I was able to “extract” the cells from the taste bud after patch-clamp recording. These Leaky cells were elongated and possessed an apical process. In one cell, this process split in two branches (data not shown). Cm measurements indicated that Leaky cells have a larger membrane surface area than the other taste cells (Fig. 2). Thus Leaky cells might have lateral cytoplasmic projections, not visible at the light microscopic level, similar to those characterizing type I (dark) cells (Pumplin et al. 1997). Until recordings are correlated with cell identification at the electron microscopic level, this interpretation will remain speculation. Interestingly, a recent study by Lawton et al. (2000) indicates that the glial glutamate transporter GLAST occurs in the plasma membrane of dark cells, suggesting that these cells may play a glialike role in glutamate handling inside taste buds.

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