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Basolateral K^+ Conductance Establishes Driving Force for Cation Absorption by Outer Sulcus Epithelial Cells

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Abstract. Outer sulcus epithelial cells were recently found to actively reabsorb cations from the cochlear luminal fluid, endolymph, via nonselective cation channels in the apical membrane. Here we determined the transport properties of the basolateral membrane with the whole-cell patch clamp technique; the apical membrane contributed insignificantly to the recordings. Outer sulcus epithelial cells exhibited both outward and inward currents and had a resting membrane potential of −90.4 ± 0.7 mV ($n = 78$), close to the Nernst potential for K^+ (−95 mV). The reversal potential depolarized by 54 mV for a tenfold increase in extracellular K^+ concentration with a K^+/Na^+ permeability ratio of 36. The most frequently observed K^+ current was voltage independent over a broad range of membrane potentials. The current was reduced by extracellular barium (10^{-5} to 10^{-3} M), amiloride (0.5 mM), quinine (1 mM), lidocaine (5 mM) and ouabain (1 mM). On the other hand, TEA (20 mM), charybdotoxin (100 nM), apamin (100 nM), glibenclamide (10 μM), 4-aminopyridine (1 mM) and gadolinium (1 mM) had no significant effect. These data suggest that the large K^+ conductance, in concert with the Na^+,K^+-ATPase, of the basolateral membrane of outer sulcus cells provides the driving force for cation entry across the apical membrane, thereby energizing vectorial cation absorption by this epithelium and contributing to the homeostasis of endolymph.

Key Words: Whole-cell patch clamp — Gerbil — Basolateral membrane

Introduction

The endolymphatic fluid of the cochlear lumen has a high $[K^+]$ of about 150 mM and a low $[Na^+]$ of about 1 mM; this unusual extracellular ion composition is necessary for transduction of sound into nerve impulses by the sensory hair cells (Marcus, 2001). The epithelium bounding the lumen is comprised of about 12 different cell types occurring in distinct microdomains and most contribute to the ionic homeostasis of endolymph (*refer to* Fig. 12A). The marginal cells of the stria vascularis secrete K^+ into the lumen (Konishi, Hamrick & Walsh, 1978; Wangemann, Liu & Marcus, 1995) and the outer sulcus epithelial cells in the upper turns of the cochlea contribute to endolymph homeostasis by absorbing both Na^+ and K^+ (Marcus & Chiba, 1999). This absorption was found to occur via nonselective cation channels in the apical membrane (Chiba & Marcus, 2000).

Transepithelial absorption of cations from endolymph by outer sulcus epithelial cells requires ion transporters in the basolateral membrane that provide a driving force for cation entry across the apical membrane and that remove the cations from the cell cytosol. We applied whole-cell patch-clamp techniques to determine the primary transport pathways and to assess their pharmacologic profiles. Our findings support a cell model in which the basolateral membrane contains a dominant K^+ conductance and a Na^+,K^+-ATPase ("Na^+-pump").

Materials and Methods

Preparation

The tissue preparation of outer sulcus epithelial cells was described previously (Marcus & Chiba, 1999). Briefly, gerbils (4–10 weeks old) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and decapitated using a protocol approved by the Institutional Animal Care and Use Committee. The temporal bone was removed and the spiral ligament with outer sulcus epithelial cells from the third turn and apex was dissected at 4°C in solution B2 (Table 1). The spiral ligament was folded into a loop with the apical membrane of outer sulcus epithelial cells facing outward and transferred to a recording chamber mounted on the stage of an inverted microscope (Nikon, Diaphot) such that

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Table 1. Composition of bath and pipette solutions (mM)

Solution	Bath						Pipette
	B1	B2	B3	B4	B5	B6	P1
NaCl	150						10
Kcl	3.6					3.6	30
$MgCl_2$	1					1	1
$Mg(SO_4)_2$		1	1	1	1		
$CaCl_2$	0.7					0.7	
Glucose	5	5	5	5	5		
HEPES	10	10	10	10	10	10	10
EGTA							1
Na-gluconate		150	141.6	108.6	3.6		
K-gluconate		3.6	12	45	150		110
Ca-gluconate		4	4	4	4		
NMDG-Cl						150	
pH	7.4	7.4	7.4	7.4	7.4	7.4	7.2

giga-ohm seals could be formed between the patch pipette and the apical membrane under visual control.

The outer sulcus epithelial structure varies with its location in the cochlea (Duvall, 1969; Spicer & Schulte, 1996). We used outer sulcus cells from the third turn and above, where the apical membrane of these cells faces the endolymph. In the lower turns, outer sulcus epithelial cells are covered by Claudius cells and have no direct contact with endolymph.

Solutions and Drugs

The compositions of solutions used are listed in Table 1. Barium chloride was from Fisher Scientific (Fair Lawn, NJ), 4-aminopyridine (4-AP) was from Aldrich (Milwaukee, WI) and amiloride, ouabain, quinine, lidocaine, glibenclamide, charybdotoxin, apamin, tetraethylammonium chloride (TEA-Cl) and gadolinium (Gd^{3+}) were from Sigma (St. Loius, MO). Nystatin (200 μg/ml; Sigma) was dissolved with sonification in the pipette solution (solution P1, Table 1) just before use. All other chemicals for the electrophysiological experiments were purchased from Sigma or Fluka (Ronkonkoma, NY). Ba^{2+}, charybdotoxin, Gd^{3+} and TEA-Cl were dissolved directly into control solution. Apamin was predissolved in 0.05 M acetic acid (5 mg/ml) and the final concentration of acetic acid was less than 0.1%. Amiloride, ouabain, 4-AP, quinine, lidocaine and glibenclamide were predissolved in dimethylsulfoxide (DMSO) to a final DMSO concentration of <0.1%.

Electrophysiological Recordings

Ionic currents were recorded with a Dagan 3900 (Minneapolis, MN) or Axon Instruments (Foster City, CA) 200A patch-clamp amplifier under voltage or current clamp in the conventional and perforated-patch whole-cell patch-clamp configurations; measurements were at 37°C. Patch pipettes were made from Corning 7052 glass with a 2-stage puller and a microforge, and the resistance was 3–6 MΩ. High-resistance seals (2–10 GΩ) were made to the apical membrane. The zero-current potential was measured in the current-clamp mode immediately (within 1 min) after the whole-cell configuration was made. After establishing a stable whole-cell configuration, the access resistance (conventional whole-cell: 12.1 ± 0.8 MΩ (average ± SEM), n = 72; perforated whole-cell: 27.4 ± 3.4 MΩ, n = 12) and the membrane capacitance (conventional whole-cell: 2.8 ± 0.2 pF, n = 72, perforated whole-cell: 2.2 ± 0.5 pF, n = 12) were measured with the circuitry in the patch-clamp amplifier. Capacitance was compensated fully and resistance compensated by 74% (mean). Command voltages were corrected for the liquid junction potential between the pipette and the bath solutions. Currents were passed through an 8-pole Bessel low-pass filter with a 1 kHz cutoff and the sampling rate was 2 kHz. Digitized data were analyzed using pClamp software (version 6, Axon Instruments). Voltage protocols are given in the Results. Currents were not corrected for "leakage" since there was no pronounced voltage dependence that could be utilized for separating the predominant K^+ current from any others that may have been present; however, the high K^+ selectivity of the cell suggests an insignificant contribution of other "leak" pathways.

Data Presentation and Statistics

All data in the text are given as mean ± SEM. The number of observations (n) is equal to the number of cells. Student's t-test of paired and unpaired samples, as appropriate, was applied and a level of $P < 0.05$ taken as statistically significant.

Results

Resting Membrane Potentials and Cell Capacitance of Outer Sulcus Epithelial Cells

The small cell capacitance measured is consistent with recordings from single, uncoupled epithelial cells. The average cell capacitance was 2.7 ± 0.2 pF, n = 84. In the upper cochlear turns, the area of cell bodies is 375 μm^2 (Spicer & Schulte, 1996). Since the expected capacitance of a simple sphere having an area of 300 μm^2 and a membrane of 1 $\mu F/cm^2$ would be 3 pF, the cell

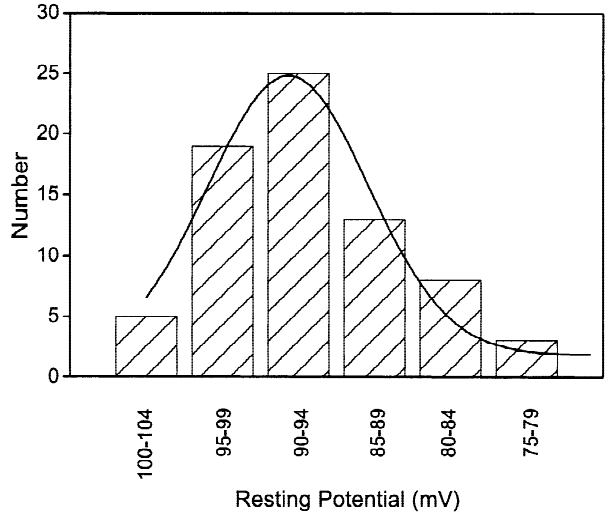

Fig. 1. Histogram of the resting potential of outer sulcus cells (n = 73). Nonlinear best fit Gaussian distribution shown with solid line. Resting potentials were obtained from the reversal potential of macroscopic current in voltage clamp and/or from the zero current voltage in current clamp.

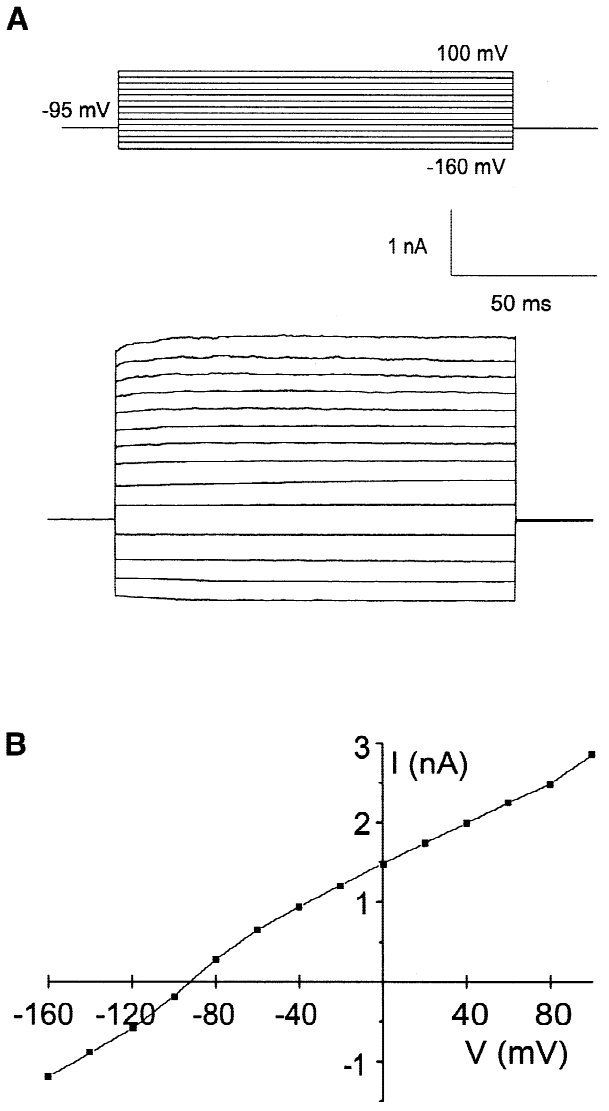

Fig. 2. Representative conventional whole-cell recording from an outer sulcus epithelial cell. (*A*) Voltage step protocol and response; (*B*) Steady-state *I-V* relationship obtained from end points of each tested voltage pulse. Current reversed beyond −90 mV. High-K^+ pipette (solution P1); Low-K^+ bath (solution B1).

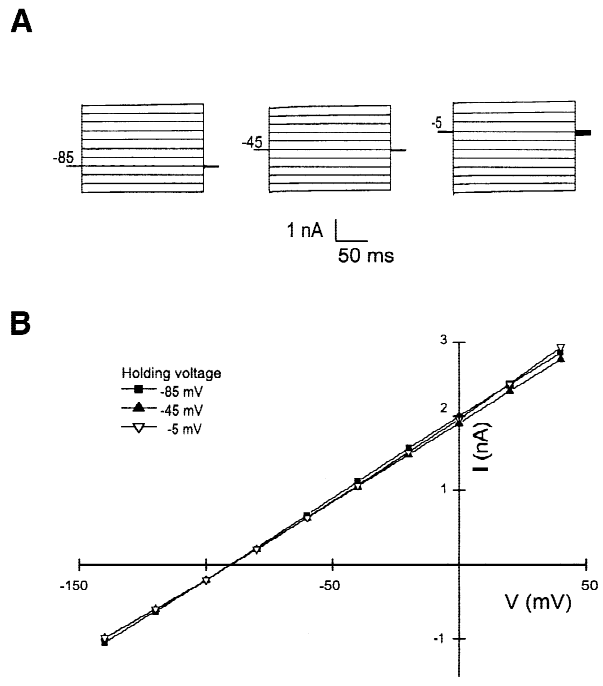

Fig. 3. Steady-state *I-V* relationships determined with the cells at different holding potentials. (*A*) The cell was held at −85 or −45 or −5 mV, stepped for 200 msec to voltages from −140 mV to +40 mV in 20-mV increments and then returned to each initial holding potential. (*B*) Plots of steady-state *I-V* relationships obtained from each current trace shown in *A.* The overlapping *I-V* relationships indicate no dependence on holding potential.

capacitance obtained in the present study was close to the expected value of a single isolated cell with little basolateral infolding.

A histogram of the resting potentials of outer sulcus epithelial cells obtained immediately after establishing the conventional whole cell configuration is shown in Fig. 1 and displays a unimodal Gaussian distribution, suggesting that our sample comes from one population of cells. The average resting potential was −90.4 ± 0.7 mV ($n = 78$) in these conventional-patch cells and was slightly less in nystatin-perforated patch-clamped cells (−84.1 ± 2.4 mV, $n = 15$). The high negative resting potential suggests that the membrane is mainly K^+ conductive since the Nernst potential for K^+ in these recordings was about −95 mV. Results are from conventional whole-cell patch recordings unless otherwise stated.

Membrane Currents of Outer Sulcus Epithelial Cells

Both inward and outward currents were recorded in response to hyperpolarization and depolarization steps from a holding potential of −95 mV. A representative example is shown in Fig. 2; similar results were observed in 45 cells. Currents changed rapidly following the onset of the voltage step and remained stable and were quasi-linear over a range of −160 to 100 mV, suggesting that these currents were not voltage dependent. The steady-state current-voltage relationship (*I-V*) is shown in Fig. 2*B.* In order to test the voltage dependency of these currents, we recorded currents over a range of holding potentials. A representative example is shown in Fig. 3. The cell was held at −85, −45 or −5 mV and then stepped from −140 to 40 mV in increments of 20 mV, returning to the initial holding voltage following each step. *I-V* relationships of each condition were nearly identical, indicating no dependence of these currents on the holding voltage.

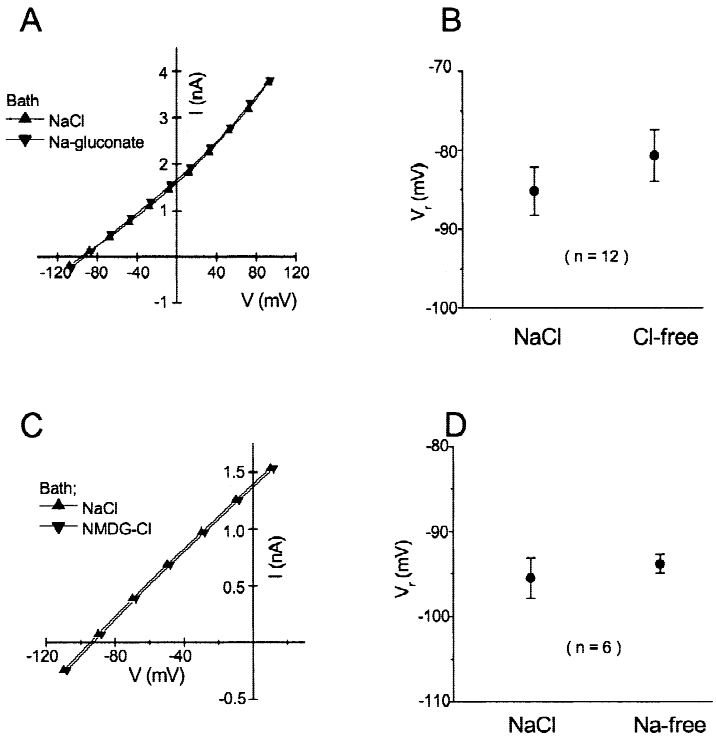

Fig. 4. Ion substitutions for Cl^- and Na^+ have no effect on whole cell currents. (*A*) Representative *I-V* relationship in the presence and absence of Cl^-. (*B*) Summary of resting membrane potential changes in experiment *A*. (*C*) Representative *I-V* relationship in the presence and absence of Na^+. (*D*) Summary of resting membrane potential changes in experiment *C*. (*B*) and (*D*): mean ± SEM.

The ion selectivity of the currents was investigated further by determining reversal potentials from the *I-V* relationships after ion substitutions in the bath. Replacement of 150 mM NaCl (solution B1) with 150 mM Na-gluconate (solution B2) caused a small but significant shift of reversal potential V_r from −85.2 ± 3.0 to −80.7 ± 3.3 mV, $n = 12$ (Fig. 4*A*,*B*). This shift is in the opposite direction from that expected for a chloride conductance and may represent an effect of gluconate on other membrane conductances. Replacement of 150 mM NaCl (solution B1) with 150 mM NMDG-Cl (solution B6) in paired experiments had no significant effect on the reversal potential (−95.5 ± 2.4 to −93.8 ± 1.1 mV, $n = 6$), demonstrating that outer sulcus epithelial cells had no significant Na^+ or nonselective cation conductance under control conditions (Fig. 4*C*,*D*).

K^+ Selectivity

The selectivity of the whole-cell current for K^+ was examined by measuring the shift in reversal potential by different external K^+ concentrations. When the external K^+ concentration $[K^+]_o$ was increased from 3.6 mM to 12, 45 and 150 mM, the reversal potential was depolarized (Fig. 5*A*). The reversal potential of the steady-state current was plotted against the $[K^+]_o$ (Fig. 5*B*). The mean reversal potentials in 3.6, 12, 45 and 150 mM $[K^+]_o$ were −87.5 ± 3.0 mV ($n = 6$), −65.2 ± 1.1 mV ($n = 6$), −34.5 ± 0.7 mV ($n = 6$) and −5.8 ± 0.4 mV ($n = 5$), respectively. The slope of the shift of reversal potential was 54.1 mV per decade of K^+ (from 12 to 150 mM $[K^+]_o$), slightly less than the slope predicted by the Nernst equation at 37°C (61 mV per decade of $[K^+]_o$). The relationship between the reversal voltage and $[K^+]_o$ could be approximated by the Goldman-Hodgkin-Katz voltage equation, which yielded a permeability ratio for K^+/Na^+ of 36. Similar results were obtained with perforated-patch recordings: slope of 47.7 mV per decade of K^+ (from 12 to 150 mM $[K^+]_o$) and a permeability ratio for K^+/Na^+ of 16 ($n = 3$ to 6). These results demonstrated that the steady-state current was highly selective for K^+.

Ion Channel Inhibitors: Active

We tested the effects of several ion channel inhibitors on V_r, conductance (G) and currents (I) at a test voltage of −28 or −25 mV in order to assess the pharmacological properties of these K^+ selective whole-cell currents. Changes in recorded currents occurred with a time course approximating the kinetics of fluid exchange in the bath.

Barium

The effects of Ba^{2+} on whole-cell currents are shown in Fig. 6. Ba^{2+} significantly decreased the current and conductance and depolarized the reversal potential at all concentrations tested (10^{-5}, 10^{-4} and 10^{-3} M). The cur-

rent was decreased by 22.5 ± 3.5%, 50.6 ± 4.8% and 79.4 ± 4.3% and the conductance by 15.9 ± 3.1%, 28.4 ± 5.6% and 34.9 ± 9.9% ($n = 6$) by 10^{-5}, 10^{-4} and 10^{-3} M Ba^{2+}. The reversal voltage was depolarized by 6.3 ± 0.7 mV, 21.7 ± 1.2 mV and 44.2 ± 2.3 mV ($n = 6$).

Quinine

The effects of quinine (1 mM) on whole-cell currents are shown in Fig. 7. Quinine significantly decreased the current and conductance and depolarized the reversal potential. The current was decreased by 79.0 ± 3.7%, but the conductance was not significantly altered (−4.7 ± 6.3%, $n = 7$). The reversal voltage was depolarized by 45.9 ± 1.2 mV ($n = 7$).

Lidocaine

The effects of lidocaine (5 mM) on whole-cell currents were similar to those of quinine and are shown in Fig. 8. Lidocaine significantly decreased the current and conductance and depolarized the reversal potential. The current was decreased by 32.8 ± 3.8% but the conductance was not significantly altered (−5.7 ± 5.8%, $n = 7$). The reversal potential was depolarized by 16.7 ± 1.1 mV ($n = 7$).

Amiloride

The effects of amiloride on whole-cell currents are shown in Fig. 9. Amiloride significantly and reversibly decreased the current and conductance and depolarized the reversal potential at both concentrations tested (0.5 and 1 mM). The current was decreased by 52.1 ± 7.3% and 74.3 ± 6.0% and the conductance by 29.1 ± 7.6% and 37.4 ± 7.7% ($n = 7$) by 0.5 and 1 mM amiloride. The reversal potential was depolarized by 20.1 ± 4.1 mV and 35.6 ± 5.5 mV ($n = 7$). These experiments were repeated in the absence of bath Na^+ (solution B6) in order to test for the involvement of Na^+/H^+ exchange in this response to amiloride. The results were similar to those obtained in the presence of bath Na^+. Amiloride significantly decreased the current and conductance and depolarized the reversal potential at both concentrations tested (0.5 and 1 mM; Fig. 10). The current was decreased by 25.4 ± 5.4% and 45.9 ± 5.2% and the conductance by 18.1 ± 7.5% and 28.8 ± 6.6% ($n = 7$) by 0.5 and 1 mM amiloride. The reversal potential was depolarized by 10.7 ± 0.6 mV and 20.3 ± 1.1 mV ($n = 7$).

ION CHANNEL INHIBITORS: INACTIVE

We tested additional ion channel blockers to further characterize these currents. Gadolinium (1 mM; blocker of nonselective cation channels), TEA (20 mM; blocker of several types of K^+ channels), 4-AP (1 mM; as blocker of voltage-dependent K^+ channels), charybdotoxin (100 nM; blocker of maxi-K^+ (BK) channels), apamin (100 nM; blocker of small-conductance Ca^{2+}-dependent K^+ channel) and glibenclamide (10 μM; blocker of ATP-sensitive K^+ channels) had no significant effects on current, conductance or reversal potential of these K^+ currents (Table 2).

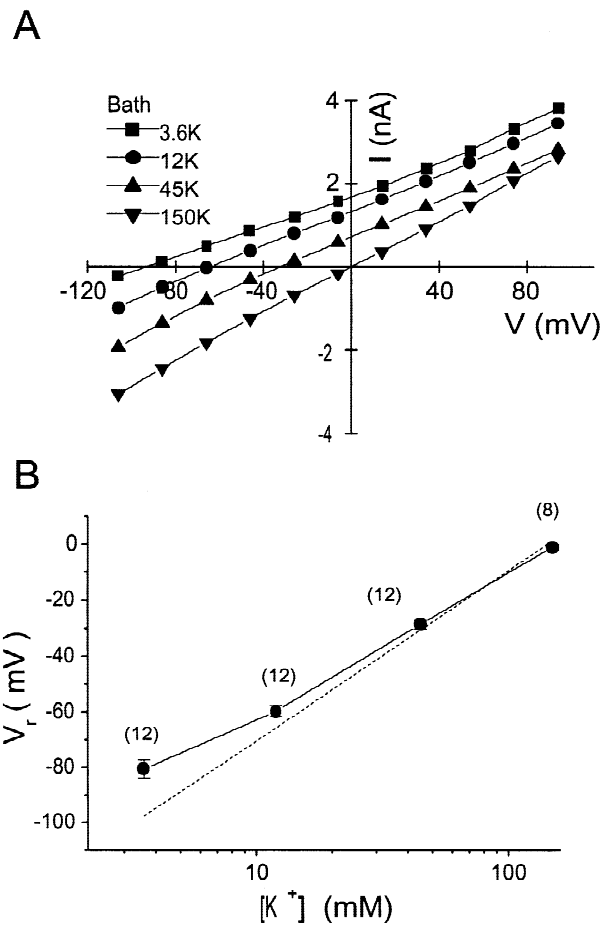

Fig. 5. Effect of K^+ concentration on steady-state current-voltage relationship. (*A*) Steady-state current-voltage relationships taken at 200 msec after the onset of each voltage pulse in 3.6, 12, 45 and 150 mM K^+ bath solution. (*B*) Mean reversal potential plotted against external K^+ concentration (mean ± SEM). The dashed line is the predicted Nernst equation at 37°C.

Na^+,K^+-ATPASE INHIBITOR

Ouabain, an inhibitor of Na^+,K^+-ATPase (1 mM), significantly decreased the current and conductance and depolarized the reversal potential (Fig. 11). The current was decreased by 17.0 ± 3.8% and the conductance by 7.6 ± 2.7% ($n = 7$). The reversal potential was depolarized by 8.7 ± 1.2 mV ($n = 7$).

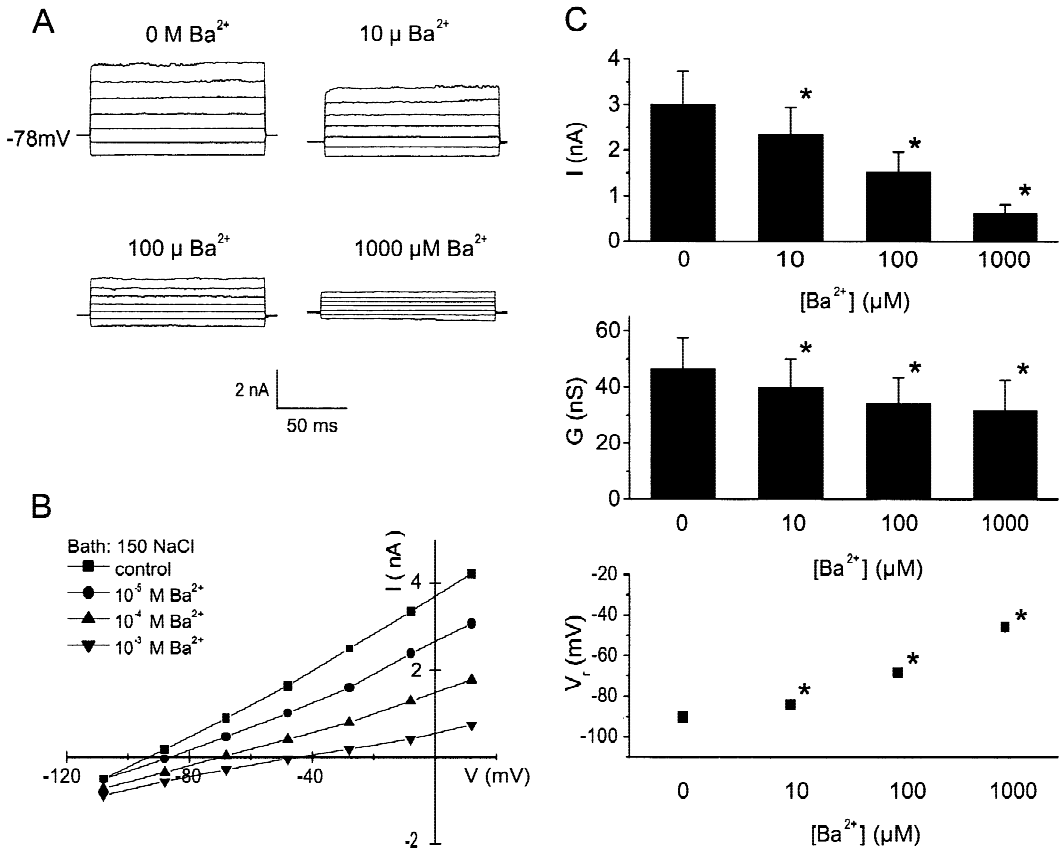

Fig. 6. Effect of Ba^{2+} on whole-cell currents. (*A*) Whole-cell recordings in the presence or absence of Ba^{2+} (10, 100, 1000 μM). Currents were elicited by 20 mV incremental voltage steps from a holding voltage of −78 mV. (*B*) Steady-state current-voltage relationships derived from recordings in *A*. (*C*) Summary of Ba^{2+} effect on steady-state whole cell currents (mean ± SEM, $n = 6$). *I*, membrane current at test voltage of −28 mV; *G*, conductance near reversal potential (V_r). *, $P < 0.05$.

Discussion

The outer sulcus epithelium was recently found to participate in endolymph homeostasis by absorption of monovalent cations (Marcus & Chiba, 1999). The cations cross the apical membrane by electrochemical diffusion through nonselective cation channels (Chiba & Marcus, 2000). Those findings can be combined with the present results to formulate a cell model of vectorial ion transport by this epithelium (Fig. 12). The electrical driving force across the apical membrane is the sum of the endocochlear (transepithelial) potential of about +80 mV and the basolateral membrane potential of about −90 mV or a total of 170 mV. If intracellular Na^+ is maintained by the Na^+-pump at about 10 mM and intracellular K^+ at 150 mM, there will be chemical components to the driving force of −60 mV for Na^+ and 0 mV for K^+ since endolymphatic Na^+ is about 1 mM and K^+ about 150 mM in the cochlea. The inward driving forces across the apical membrane are therefore quite large and are 110 mV for Na^+ and 170 mV for K^+. The role of the Na^+-pump is therefore to maintain the intracellular ion composition, removing Na^+ that enters the cell across the apical membrane and keeping K^+ high to generate the large membrane potential across the basolateral K^+ conductance.

Properties measured in the whole-cell configuration originate from both the apical and basolateral membranes. However, the nonselective cation channels in the apical membrane did not contribute significantly to our recordings; gadolinium is a potent blocker of those channels from both the extracellular and cytosolic sides of the cell membrane (Chiba & Marcus, 2000) but had no significant effect on the whole-cell currents. The basolateral membrane surface area is far greater than that of the apical membrane; the greater area combined with an apparently high basolateral K^+ channel density led to a domination of the whole-cell recordings by the basolateral K^+ conductance. Contributions from Cl^- or Na^+ channels were also minimal or nonexistent. Replacement of bath Cl^- by the impermeant anion gluconate led to a small depolarization of membrane potential that was not consistent with a Cl^- conductance. This is consistent with previous findings that neither the transepithelial current nor the cell volume were affected by Cl^- removal

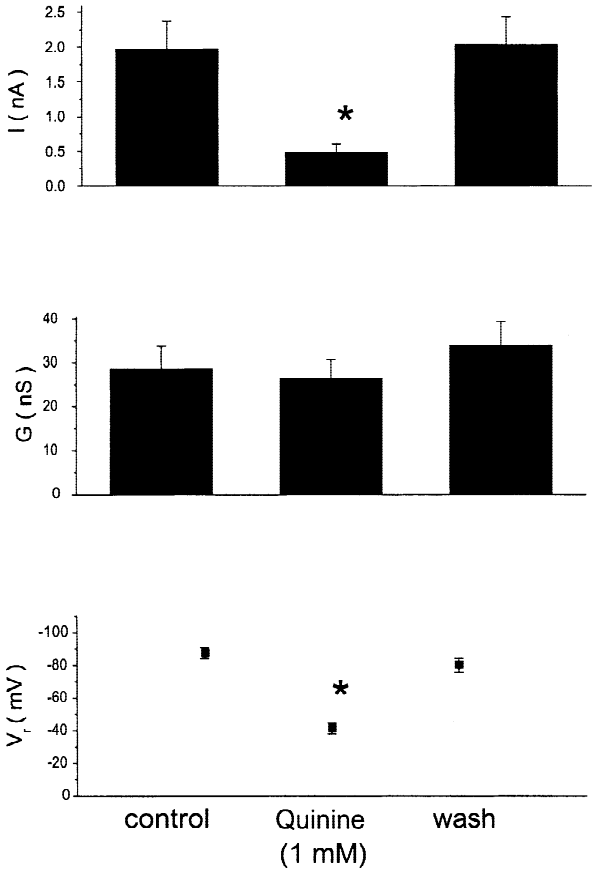

Fig. 7. Summary of effect of quinine (1 mM) on whole-cell currents (mean ± SEM, $n = 7$). *I*, membrane current at test voltage of −25 mV; *G*, conductance near reversal potential (V_r). *, $P < 0.05$.

(Marcus & Chiba, 1999). Absence of significant Na^+ conductance was demonstrated by the finding that replacement of Na^+ by the impermeant cation NMDG had no significant effect on membrane potential.

Pharmacological Characterization of Whole-Cell Currents

The whole-cell current was significantly inhibited by Ba^{2+}, quinine, lidocaine, amiloride and ouabain. Known membrane targets of these agents are discussed below. The currents were not affected by charybdotoxin, apamin, glibenclamide or TEA. These results suggest that the basolateral channels carrying this K^+ current were not the Ca^{2+}-dependent maxi-K^+ ("big") channel (inhibited by extracellular charybdotoxin and TEA) or "small" K^+ channels (inhibited by the bee venom apamin) or ATP-sensitive K^+ channels (inhibited by glibenclamide). This pharmacologic profile is quite similar to the K^+ conductance in the basolateral membrane of vestibular transitional cells (Wangemann & Marcus, 1989; Wangemann & Shiga, 1994).

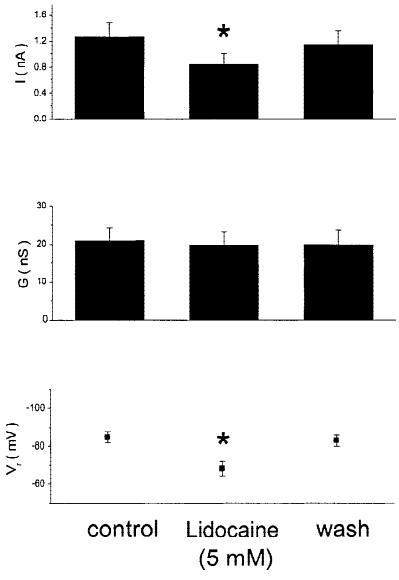

Fig. 8. Summary of effect of lidocaine (5 mM) on whole-cell currents (mean ± SEM, $n = 6$). *I*, membrane current at test voltage of −25 mV; *G*, conductance near reversal potential (V_r). *, $P < 0.05$.

Barium

Ba^{2+} blocked whole-cell K^+ currents in a monotonic dose-dependent manner between 10 and 1000 μM in outer sulcus epithelial cells. Ba^{2+} is a well-established blocker of many epithelial K^+ channels (Farrugia & Rae, 1993; Tao et al., 1994; Kotera & Brown, 1994) and the sensitivity to Ba^{2+} was similar to that found for Kir4.1 channels in cochlear intermediate cells (Takeuchi & Ando, 1999) and for the K^+ conductance found in vestibular transitional cells (Wangemann & Marcus, 1989). The depolarization of membrane voltage was likely due to a minor population of channels with a permeability to Na^+ and this minor population contributed more to the membrane potential as the predominant K^+ channels became pharmacologically "removed" by the K^+ channel blockers, as observed in vestibular transitional cells (Wangemann & Marcus, 1989).

Quinine

Quinine is known to block K^+ channels in several epithelial cell types (Sullivan et al., 1990; Takeuchi, Marcus & Wangemann, 1992; Osipenko, Evans & Jurney, 1997). In the present study, quinine strongly depolarized the reversal potential, but did not significantly reduce the conductance of whole-cell currents. This observation suggests that in addition to blocking the K^+ conductance, quinine may have also stimulated or induced another conductance, such as a nonselective cation conductance

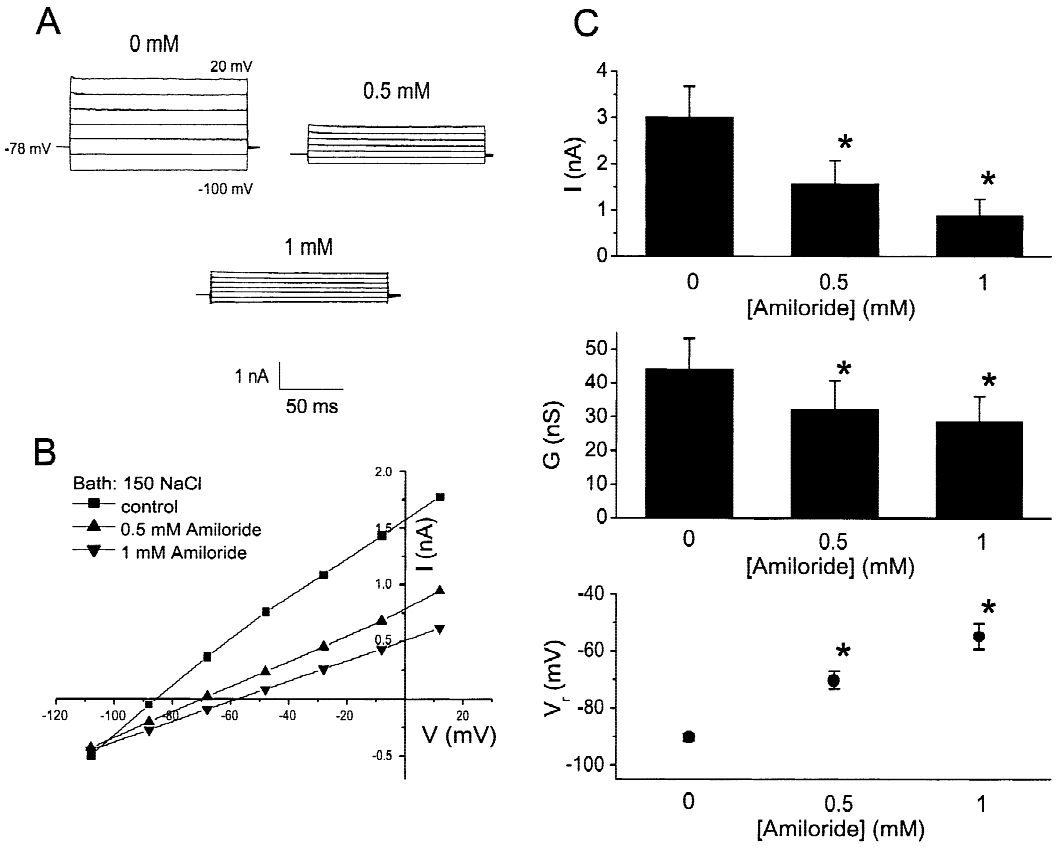

Fig. 9. Effect of amiloride in the presence of bath Na^+ on whole-cell currents. (*A*) Whole-cell recordings in the presence or absence of amiloride (0.5, 1 mM). Currents were elicited by 20 mV incremental voltage steps from a holding voltage of −78 mV. (*B*) Steady-state current-voltage relationships derived from recordings in *A*. (*C*) Summary of amiloride effect on steady-state whole cell currents (mean ± SEM, $n = 7$). *I*, membrane current at test voltage of −28 mV; *G*, conductance near reversal potential (V_r). *, $P < 0.05$.

as found in vertebrate taste receptor cells (Tsunenari et al., 1996).

Lidocaine

Lidocaine is known as a reversible blocker of basolateral K^+ channels in several epithelia (Van, 1986; Dawson, Van & Helman, 1988; Illek, Fisher & Clauss, 1990) and in the apical membrane of vestibular dark cells (Takeuchi et al., 1992). In contrast to our findings, the block in turtle colon was only on osmotically-activated K^+ conductance (Dawson et al., 1988). Similar to quinine, lidocaine strongly depolarized the reversal potential, but did not significantly reduce the conductance of whole-cell currents. Again, this observation suggests that in addition to blocking the K^+ conductance, lidocaine may have also stimulated or induced another conductance.

Ouabain

The finding that ouabain depolarized the membrane potential and significantly reduced both K^+ currents and conductance demonstrate the presence of a Na^+,K^+-ATPase in outer sulcus epithelial cells. This is consistent with the immunohistochemical observation that Na^+,K^+-ATPase is present in the lateral membrane of these cells (Nakazawa, Spicer & Schulte, 1995). Similar electrophysiologic results were obtained in vestibular transitional cells (Wangemann & Marcus, 1989), cultured pericytes (Wagner & Wiederholt, 1996) and amphibian proximal tubule (Messner et al., 1985).

Depolarization by ouabain can be explained by two components, as found in other cells (Wangemann & Marcus, 1989). The magnitude of depolarization observed in our experiments was too large to be explained solely by inhibition of the pump current. It has been found in many cells that depolarization by ouabain occurs in two phases, the first of which is caused by inhibition of pump current and the second by a redistribution of K^+ across the cell membrane. In the second phase, K^+ exits the cell during the inhibition of Na^+,K^+-ATPase, leading to a lower electromotive force for K^+. If the K^+ conductance were due to the presence of the ATP-inhibited K_{ATP} channel (Kir6.2), the results could be explained by an increase in cellular ATP concentration

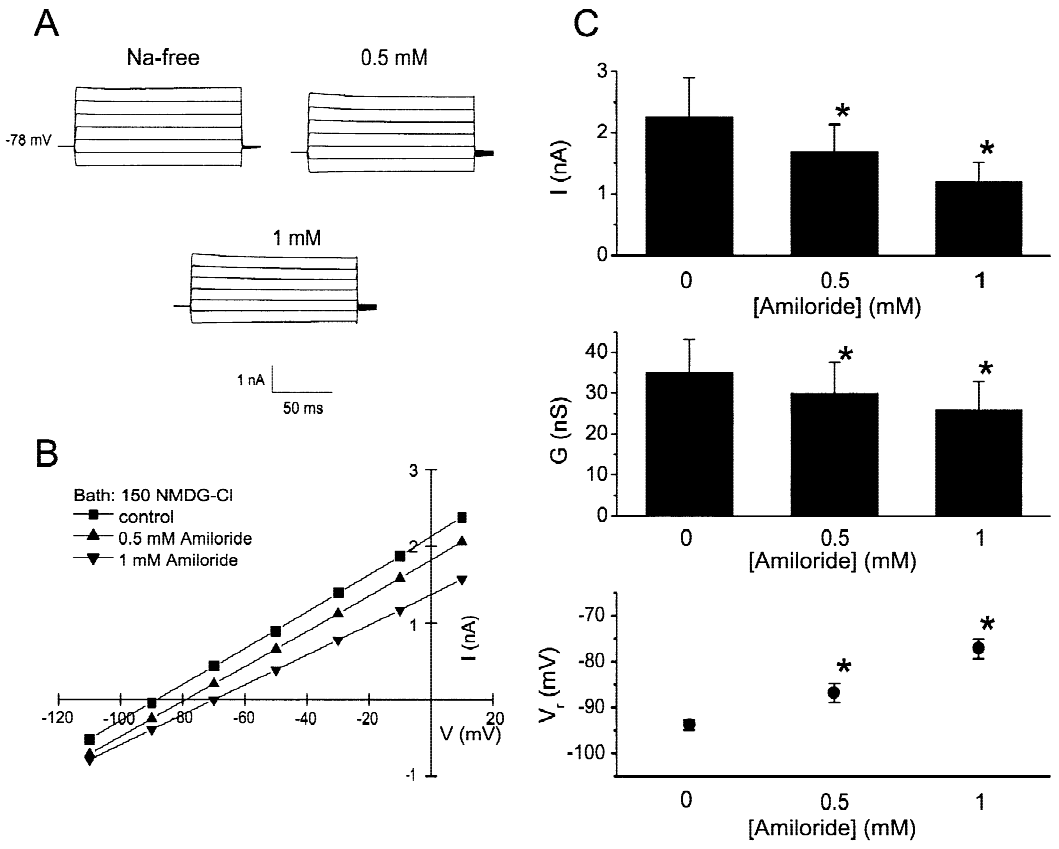

Fig. 10. Effect of amiloride in the absence of bath Na^+ on whole-cell currents. (*A*) Whole-cell recordings in the presence or absence of amiloride (0.5, 1 mM). Currents were elicited by 20 mV incremental voltage steps from a holding voltage of −78 mV. (*B*) Steady-state current-voltage relationships derived from recordings in *A*. (*C*) Summary of amiloride effect on steady-state whole cell currents (mean ± SEM, $n = 6$). *I*, membrane current at test voltage of −28 mV; *G*, conductance near reversal potential (V_r). *, $P < 0.05$.

Table 2. Ion channel blockers causing no effect on whole-cell currents

Blocker	Concentration	V_r mV	*G* nS	*I* (−25 mV) nA	No. of Cells
TEA	0	−89.9 ± 1.6	34.7 ± 7.4	2.44 ± 0.64	7
	20mM	−89.0 ± 1.5	32.6 ± 7.2	2.20 ± 0.57	7
4-AP	0	−91.6 ± 2.1	38.1 ± 16.8	1.91 ± 0.80	5
	1mM	−91.4 ± 2.2	36.7 ± 16.9	1.92 ± 0.78	5
CTX	0	−86.8 ± 3.0	68.5 ± 23.4	2.83 ± 0.67	5
	100 nM	−88.6 ± 2.7	65.7 ± 22.8	2.94 ± 0.72	5
Apamin	0	−88.7 ± 3.6	52.4 ± 14.0	2.36 ± 0.47	6
	100 nM	−87.5 ± 4.0	50.1 ± 12.8	2.35 ± 0.48	6
Glibenclamide	0	−91.0 ± 2	35.6 ± 16.7	1.87 ± 0.77	5
	10 μM	−91.2 ± 2.2	34.9 ± 15.8	1.81 ± 0.74	5
Gadolinium	0	−87.4 ± 2.3	25.6 ± 4.9	1.76 ± 0.35	7
	1mM	−87.2 ± 2.0	23.9 ± 4.5	1.60 ± 0.31	7

when the ATPase was inhibited. However, Kir6.2 is known to be blocked by the sulphonylurea glibenclamide (Ruppersberg, 2000), which had no effect in the present experiments.

Amiloride

Our results with amiloride are most simply interpreted as demonstrating a direct inhibition of the basolateral K^+

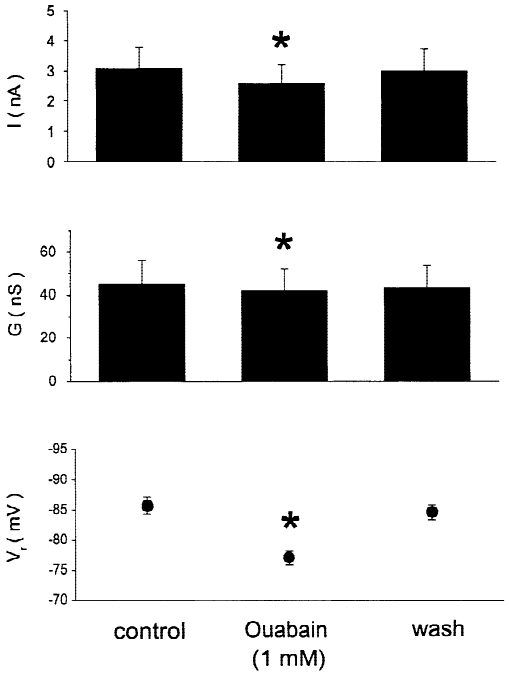

Fig. 11. Summary of effect of ouabain (1 mM) on whole-cell currents (mean ± SEM, n = 7). *I*, membrane current at test voltage of −25 mV; *G*, conductance near reversal potential (V_r). *, $P < 0.05$.

conductance. Amiloride in micromolar concentrations is a well-known blocker of epithelial Na^+ channels (Garty & Palmer, 1997) and some nonselective cation channels (Marunaka et al., 1994; Ono et al., 1994; Chiba & Marcus, 2000). However, neither epithelial Na^+ channels nor nonselective cation channels contributed significantly to the whole-cell conductance since replacement of external Na^+ with NMDG caused no observable change in the reversal potential. Further, block of either of these types of conductance by amiloride would be expected to cause a hyperpolarization of the cells rather than the observed depolarization. The nonselective cation channel conductance in the apical membrane (Marcus & Chiba, 1999; Chiba & Marcus, 2000) is therefore far smaller than the basolateral K^+ conductance.

Millimolar concentrations of amiloride are known to inhibit Na^+/H^+ exchange (Noël & Pouysségur, 1995), Na^+/Ca^{2+} exchange (Kaczorowski et al., 1985) and Na^+,K^+-ATPase (Soltoff & Mandel, 1983). The observation that Na^+ replacement did not mimic the effects of amiloride in causing a significant depolarization and inhibition of K^+ currents suggests that the amiloride effects were not due to inhibition of a Na^+/H^+ or Na^+/Ca^{2+} exchanger. The effect of amiloride was not mediated by inhibition of Na^+,K^+-ATPase since the inhibition of K^+ current caused by amiloride was much larger than that caused by 1 mM ouabain, a concentration sufficient to fully inhibit the enzyme in this species (Marcus, Marcus & Greger, 1987).

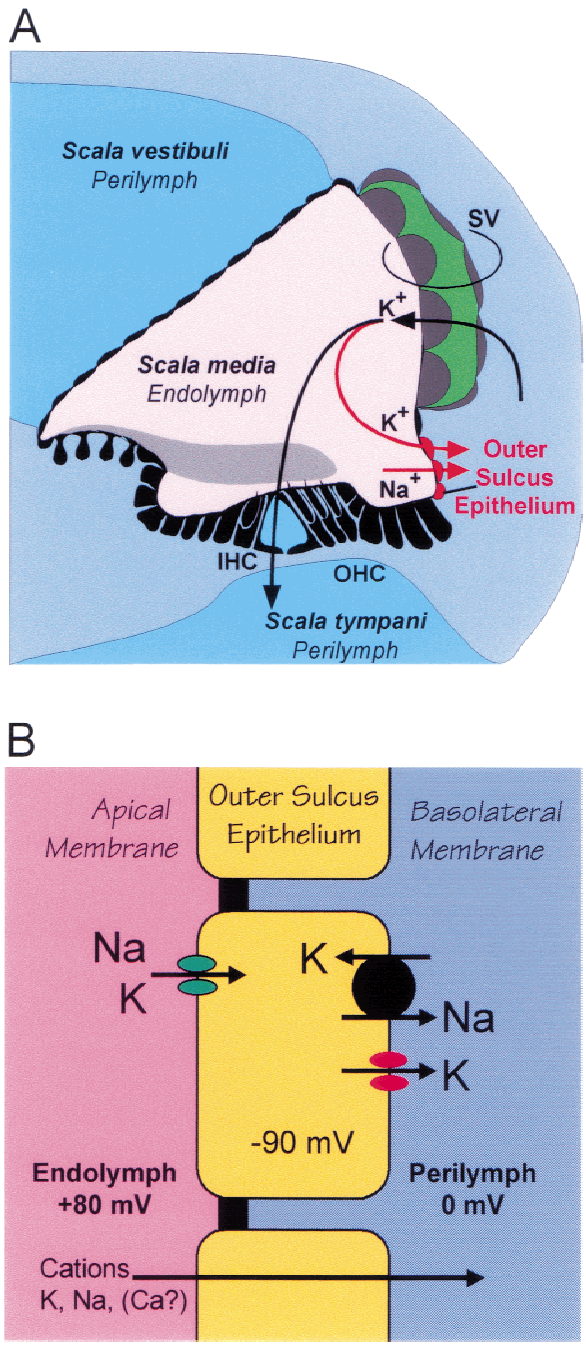

Fig. 12. Cell model of cation absorption by outer sulcus epithelial cells. (*A*) Diagram of cross section of cochlear duct, illustrating the position of the outer sulcus epithelial cells. Arrows illustrate path of secretion of K^+ by the stria vascularis (SV) and efflux through sensory inner and outer hair cells (IHC & OHC) and along with Na^+ through outer sulcus cells. (*B*) Na^+ and K^+ enter the cell across the apical membrane through nonselective cation channels. Na^+ is removed across the basolateral membrane by the Na^+,K^+-ATPase and K^+ diffuses across the basolateral K^+ channels. Endolymph is +80 mV and the cell interior −90 mV with respect to the perilymph.

A blocking action of amiloride on K^+ channels has been previously reported. Amiloride inhibited K^+ conductance in several epithelial cells, including in the proximal tubule of the mammalian kidney (Zweifach et al., 1992; Discala et al., 1992) and vestibular transitional cells (Wangemann & Shiga, 1994) and blocked a stretch-activated K^+ channel in *Lymnaea* neurons (Small & Morris, 1995). In the present experiments, amiloride was effective in depolarizing the reversal potential, although not as potent as Ba^{2+}, and was effective both in the presence and absence of bath Na^+. The mechanism of action of amiloride to inhibit the K^+ conductance in outer sulcus epithelial cells is not yet clear and may be either a direct interaction with the K^+ channels or an indirect effect. It is conceivable that amiloride acted by reducing apical Na^+ entry via the nonselective cation channels (Chiba & Marcus, 2000), which subsequently reduced the basolateral membrane Na^+,K^+-ATPase activity followed by the widely-reported concomitant reduction of the basolateral K^+ conductance (Messner et al., 1985).

Cell Model

We had shown previously that the outer sulcus epithelium contributes to the homeostasis of endolymph by absorption of cations. The present study completes the model of constitutive vectorial transport by this tissue (Fig. 12).

Two types of Ca^{2+}-dependent cation channels were identified in the apical membrane: a nonselective cation channel, which is constitutively open at the typical resting level of 10^{-7} M intracellular Ca^{2+} and a maxi-K^+ channel, which is closed in 10^{-7} M intracellular Ca^{2+} (Chiba & Marcus, 2000). The Ca^{2+} permeability of the nonselective cation channel was not determined. The nonselective cation channel in the apical membrane did not significantly contribute to the whole-cell currents reported here, due to the relatively large basolateral membrane K^+ conductance and membrane surface area.

Endolymph is +80 mV and the cell interior −90 mV with respect to the perilymph. Na^+ and K^+ enter the cell across the apical membrane through the nonselective cation channels. The net electrochemical driving force for Na^+ is about 110 mV (170 mV electrical-60 mV chemical) and that for K^+ is about 170 mV (170 mV electrical − 0 mV chemical). Na^+ is removed across the basolateral membrane by the Na^+,K^+-ATPase and K^+ diffuses across the basolateral K^+ channels.

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References

Chiba, T., Marcus, D.C. 2000. Nonselective cation and BK channels in apical membrane of outer sulcus epithelial cells. *J. Membrane Biol.* **174:**167–179

Dawson, D.C., Van, D.W., Helman, S.I. 1988. Osmotically induced basolateral K^+ conductance in turtle colon: lidocaine-induced K^+ channel noise. *Am. J. Physiol.* **254:**C165–C174

Discala, F., Hulin, P., Belachgar, F., Planelles, G., Edelman, A., Anagnostopoulos, T. 1992. Millimolar amiloride concentrations block K conductance in proximal tubular cells. *Br. J. Pharmacol.* **107:**532–538

Duvall, A.J. 1969. The ultrastructure of the external sulcus in the guinea pig cochlear duct. *Laryngoscope* **79:**1–29

Farrugia, G., Rae, J. 1993. Effect of volume changes on a potassium current in rabbit corneal epithelial cells. *Am. J. Physiol.* **264:**C1238–C1245

Garty, H., Palmer, L.G. 1997. Epithelial sodium channels: function, structure, and regulation. *Physiol. Rev.* **77:**359–396

Illek, B., Fischer, H., Clauss, W. 1990. Aldosterone regulation of basolateral potassium channels in alveolar epithelium. *Am. J. Physiol.* **259:**L230–L237

Kaczorowski, G.J., Barros, F., Dethmers, J.K., Trumble, M.J., Cragoe, E.J.J. 1985. Inhibition of Na^+/Ca^{2+} exchange in pituitary plasma membrane vesicles by analogues of amiloride. *Biochemistry* **24:**1394–1403

Konishi, T., Hamrick, P.E., Walsh, P.J. 1978. Ion transport in guinea pig cochlea. I. Potassium and sodium transport. *Acta Otolaryngol. (Stockh)* **86:**22–34

Kotera, T., Brown, P.D. 1994. Evidence for two types of potassium current in rat choroid plexus epithelial cells. *Pfluegers Arch.* **427:**317–324

Marcus, D.C. 2001. Acoustic Transduction. *In:* Cell Physiology Source Book. 3rd edition. N. Sperelakis, editor. pp 775–794. Academic Press, San Diego

Marcus, D.C., Chiba, T. 1999. K^+ and Na^+ absorption by outer sulcus epithelial cells. *Hear. Res.* **134:**48–56

Marcus, D.C., Marcus, N.Y., Greger, R. 1987. Sidedness of action of loop diuretics and ouabain on nonsensory cells of utricle: a micro-Ussing chamber for inner ear tissues. *Hear. Res.* **30:**55–64

Marunaka, Y., Tohda, H., Hagiwara, N., Nakahari, T. 1994. Antidiuretic hormone-responding nonselective cation channel in distal nephron epithelium (A6). *Am. J. Physiol.* **266:**C1513–C1522

Messner, G., Wang, W., Paulmichl, M., Oberleithner, H., Lang, F. 1985. Ouabain decreases apparent potassium-conductance in proximal tubules of the amphibian kidney. *Pfluegers Arch.* **404:**131–137

Nakazawa, K., Spicer, S.S., Schulte, B.A. 1995. Ultrastructural localization of Na,K-ATPase in the gerbil cochlea. *J. Histochem. Cytochem.* **43:**981–991

Noël, J., Pouysségur, J. 1995. Hormonal regulation, pharmacology, and membrane sorting of vertebrate Na^+/H^+ exchanger isoforms. *Am. J. Physiol.* **268:**C283–C296

Ono, S., Mougouris, T., DuBose T.D. Jr., Sansom, S.C. 1994. ATP and calcium modulation of nonselective cation channels in IMCD cells. *Am. J. Physiol.* **267:**F558–F565

Osipenko, O.N., Evans, A.M., Gurney, A.M. 1997. Regulation of the resting potential of rabbit pulmonary artery myocytes by a low threshold, O_2-sensing potassium current. *Br. J. Pharmacol.* **120:**1461–1470

Ruppersberg, J.P. 2000. Intracellular regulation of inward rectifier K^+ channels. *Pfluegers Arch.* **441:**1–11

Small, D.L., Morris, C.E. 1995. Pharmacology of stretch-activated K channels in *Lymnaea* neurones. *Br. J. Pharmacol.* **114:**180–186

Soltoff, S.P., Mandel, L.J. 1983. Amiloride directly inhibits the Na,K-ATPase activity of rabbit kidney proximal tubules. *Science* **220:**957–958

Spicer, S.S., Schulte, B.A. 1996. The fine structure of spiral ligament cells relates to ion return to the stria and varies with place-frequency. *Hear. Res.* **100:**80–100

Sullivan, S.K., Swamy, K., Greenspan, N.R., Field, M. 1990. Epithelial K channel expressed in *Xenopus* oocytes is inactivated by protein kinase C. *Proc. Natl. Acad. Sci. USA* **87:**4553–4556

Takeuchi, S., Ando, M. 1999. Voltage-dependent outward K^+ current in intermediate cell of stria vascularis of gerbil cochlea. *Am. J. Physiol.* **277:**C91–C99

Takeuchi, S., Marcus, D.C., Wangemann, P. 1992. Maxi K^+ channel in apical membrane of vestibular dark cells. *Am. J. Physiol.* **262:**C1430–C1436

Tao, Q., Rafuse, P.E., Kelly, M.E.M. 1994. Potassium currents in cultured rabbit retinal pigment epithelial cells. *J. Membrane Biol.* **141:**123–138

Tsunenari, T., Hayashi, Y., Orita, M., Kurahashi, T., Kaneko, A., Mori, T. 1996. A quinine-activated cationic conductance in vertebrate taste receptor cells. *J. Gen. Physiol.* **108:**515–523

Van, D.W. 1986. Lidocaine blockage of basolateral potassium channels in the amphibian urinary bladder. *J. Physiol.* **381:**575–593

Wagner, U., Wiederholt, M. 1996. Membrane voltage and whole-cell currents in cultured pericytes of control rats and rats with retinal dystrophy. *Curr. Eye Res.* **15:**1045–1053

Wangemann, P., Liu, J., Marcus, D.C. 1995. Ion transport mechanisms responsible for K^+ secretion and the transepithelial voltage across marginal cells of stria vascularis in vitro. *Hear. Res.* **84:**19–29

Wangemann, P., Marcus, D.C. 1989. Membrane potential measurements of transitional cells from the crista ampullaris of the gerbil. Effects of barium, quinidine, quinine, tetraethylammonium, cesium, ammonium, thallium and ouabain. *Pfluegers Arch.* **414:**656–662

Wangemann, P., Shiga, N. 1994. Ba^{2+} and amiloride uncover or induce a pH-sensitive and a Na^+ or nonselective cation conductance in transitional cells of the inner ear. *Pfluegers Arch.* **426:**258–266

Zweifach, A., Desir, G.V., Aronson, P.S., Giebisch, G. 1992. Inhibition of Ca-activated K^+ channels from renal microvillus membrane vesicles by amiloride analogs. *J. Membrane Biol.* **128:**115–122