J. Membrane Biol. 147, 255–262 (1995)

The Journal of
Membrane
Biology

The pH-sensitivity of Transepithelial K^+ Transport in Vestibular Dark Cells

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Received: 13 January 1995/Revised: 26 May 1995

Abstract. The pH-sensitivity of transepithelial K^+ transport was studied in vitro in isolated vestibular dark cell epithelium from the gerbil ampulla. The cytosolic pH (pH_i) was measured microfluorometrically with the pH-sensitive dye 2′,7′-bicarboxyethyl-5(6)-carboxyfluorescein (BCECF) and the equivalent short-circuit current (I_{sc}), which is a measure for transepithelial K^+ secretion, was calculated from measurements of the transepithelial voltage (V_t) and the transepithelial resistance (R_t) in a micro-Ussing chamber. All experiments were conducted in virtually HCO_3^--free solutions. Under control conditions, pH_i was 7.01 ± 0.04 (n = 18), V_t was 9.1 ± 0.5 mV, R_t 16.7 ± 0.09 Ωcm^2, and I_{sc} was 587 ± 30 μA/cm^2 (n = 49). Addition of 20 mM propionate$^-$ caused a biphasic effect involving an initial acidification of pH_i, increase in V_t and I_{sc} and decrease in R_t and a subsequent alkalinization of pH_i, decrease of V_t and increase of R_t. Removal of propionate$^-$ caused a transient effect involving an alkalinization of pH_i, a decrease of V_t and I_{sc} and an increase in R_t. pH_i in the presence of propionate$^-$ exceeded pH_i under control conditions. Effects of propionate$^-$ on V_t, R_t and I_{sc} were significantly larger when propionate$^-$ was applied to the basolateral side rather than to the apical side of the epithelium. The pH_i-sensitivity of I_{sc} between pH 6.8 and 7.5 was −1089 μA/(cm^2 · pH-unit) suggesting that K^+ secretion ceases at about pH_i 7.6. Acidification of the extracellular pH (pH_o) caused an increase of V_t and I_{sc} and a decrease of R_t most likely due to acidification of pH_i. Effects were significantly larger when the extracellular acidification was applied to the basolateral side rather than to the apical side of the epithelium. The pH_o sensitivity of I_{sc} between pH 7.4 and 6.4 was −155 μA/(cm^2 · pH unit). These results demonstrate that transepithelial K^+ transport is sensitive to pH_i and pH_o and that vestibular dark cells contain propionate$^-$ uptake mechanism. Further, the data suggest that cytosolic acidification activates and that cytosolic alkalinization inactivates the slowly activating K^+ channel (I_{sK}) in the apical membrane. Whether the effect of pH_i on the I_{sK} channel is a direct or indirect effect remains to be determined.

Key words: Labyrinth — Slowly activating K^+ channel — I_{sK} channel — MinK channel — pH

Introduction

Changes in the pH between −0.3 and 0.2 pH-units occur during pathological conditions of acidosis and alkalosis in the blood plasma and can be expected to occur in perilymph since the blood-perilymph barrier is permeable to many small ions (Sterkers et al., 1982, 1987). A variety of ion transport mechanisms are known to be pH-sensitive but the question whether transepithelial K^+ transport in vestibular dark cells is sensitive to changes in the extracellular or cytosolic pH has so far not been addressed. Recent observations demonstrated that vestibular dark cells and strial marginal cells have many ion transport mechanisms in common (Wangemann, Liu & Marcus, 1995; Wangemann, 1995). Indirect evidence based on measurements of the endocochlear potential during vascular perfusion suggested that transepithelial K^+ transport by strial marginal cells is sensitive to changes in the extracellular and cytosolic pH (Arakawa, Marcus & Thalmann, 1987). Based on these observations it is conceivable that transepithelial K^+ transport in vestibular dark cells is pH-sensitive as well. The aim of the present study was to determine whether changes in the cytosolic or the extracellular pH affect transepithelial K^+ transport in vestibular dark cell epithelium.

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Table 1. Solutions (in mM)

Name	1	2	3	4	5	6	7
NaCl	150.0	130.0	150.0	150.0	150.0	150.0	
KCl			3.6	3.6	3.6	3.6	150.0
Na-propionate		20.0					
K_2HPO_4	1.6	1.6					
KH_2PO_4	0.4	0.4					
Na_2HPO_4			1.71	1.43	1.13	0.57	
NaH_2PO_4			0.29	0.57	0.87	1.43	
$CaCl_2$	0.7	0.7	0.7	0.7	0.7	0.7	0.7
$MgCl_2$	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Glucose	5.0	5.0	5.0	5.0	5.0	5.0	5.0
HEPES							10.0
pH[a]	7.4	7.4	7.4	7.1	6.8	6.4	6.5–7.7[b]

[a] pH at 37°C; [b]The pH of the calibration solution was adjusted to different pH values using HCl and NaOH.

Materials and Methods

Preparation

The method of dissection of vestibular dark cell epithelium has been described earlier (Wangemann & Marcus, 1990). The procedures concerning animals reported in this study were approved by Boys Town National Research Hospital Animal Care and Use Committee. Briefly, gerbils were anesthetized with pentobarbital (50 mg/kg i.p.) and decapitated. The temporal bone containing the inner ear was removed and quickly transferred into cold (4°C) dissection solution (solution 1, Table 1). Under microscopic observation the ampullae were dissected free and a patch of epithelium containing a domain of dark cell epithelium was carefully cut out. Melanocytes in the connective tissue beneath the dark cell population aided orientation. The tissue was folded in a loop so that an optical section of vestibular dark cells exclusive of the underlying connective tissue was accessible for fluorescence measurements or microelectrode impalements under visual control. Alternatively, the tissue was left as a flat sheet for measurements in the micro-Ussing chamber.

Measurement of the Cytosolic pH (pH_i)

pH_i was measured using the fluorescent dye 2′,7′-bicarboxyethyl-5(6)-carboxyfluorescein (BCECF) loaded into cells as BCECF-acetoxymethylester (BCECF-AM) as described earlier (Wangemann & Shiga, 1994). Briefly, the folded tissue was incubated on the stage of the microscope with 7 μM BCECF-AM (in solution 1) at room temperature for about 30 min. During experiments, the preparation was alternately illuminated at wavelengths of 442 and 502 nm. The emitted light passed a 516 nm dichroic mirror and a 531 ± 10 nm band-pass filter (Omega Optical, Brattleboro, VT) and a rectangular area encompassing about 10 vestibular dark cells exclusive of the connective tissue was viewed by photomultiplier.

The optimal wavelengths for the excitation of BCECF in vestibular dark cells was determined from scans at different extracellular pH values (solution 7) in the presence of 10 μM nigericin (Fig. 1). The isosbestic point was chosen as the lower wavelength and the average of the emission maxima at pH 6.8 and pH 7.2 was chosen as the upper wavelength.

The intensity of emission in response to the two excitation wavelengths was recorded, the ratio (502/442) was computed and calibrated against pH_i using the high-K^+/nigericin method (Thomas et al., 1979). During calibration the tissue was perfused with a solution (solution 7) containing 150 mM KCl and 10 μM nigericin which was adjusted to different pH values at 37°C (Fig. 2).

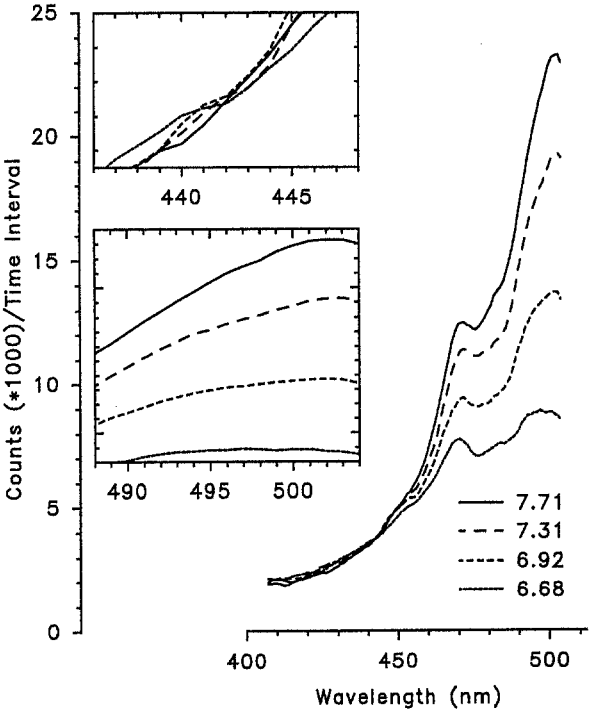

Fig. 1. Determination of the optimal excitation wavelengths. Wavelength scans of emissions were performed in the presence of different pH_i established via nigericin. Upper insert: Enlargement of the isosbestic point. Lower insert: Enlargement of the maxima.

A linear regression between the ratio (R) and pH_i was used for the calibration. To verify this method, the averaged calibration data were either fitted to a linear relationship or to the Henderson-Hasselbalch equation describing the fluorescence of BCECF $pH_i = pK_a - \log((r_{max} - R)/(R - R_{min}))$ where R_{min} and R_{max} are the minimal and maximal fluorescence. The fit to the Henderson-Hasselbalch equation yielded $R_{min} = 1.7 \pm 0.2$, $R_{max} = 9.0 \pm 0.6$, and a pK_a for BCECF in vestibular

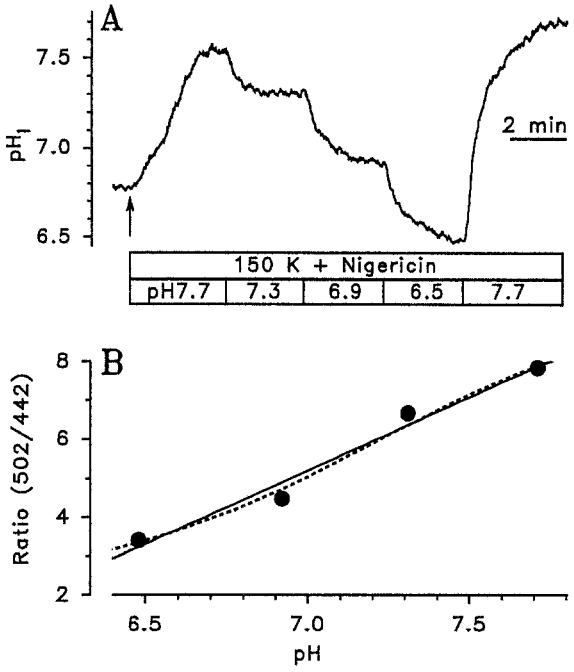

Fig. 2. Calibration of the ratio between the fluorescence intensity evoked by excitation at 442 nm and 502 nm. (*A*) Protocol performed at the end of experiments. The fluorescence ratio was measured at various pH_i which were established by the presence of 10 mM nigericin and 150 mM KCl (arrow). (*B*) The relationship between the fluorescence ratio and pH_i was fitted with a linear regression (continuous line) and with a nonlinear regression algorithm to the Henderson-Hasselbalch equation (broken line).

dark cells of 7.22 ± 0.05 (n = 5). As shown in Fig. 2*B*, there was little deviation between the two methods, suggesting that within this pH_i range a linear calibration is a valid approximation. Linear calibrations have been used for pH_i measurements in many other cells, e.g., (Weintraub & Machen, 1989; Vilella et al., 1992; Wangemann & Shiga, 1994).

Measurement of Transepithelial Parameters

For the measurement of the transepithelial voltage (V_t) and resistance (R_t) under open circuit conditions the epithelium was sealed with the apical membrane onto the aperture of the micro-Ussing chamber as described earlier (Marcus, Liu & Wangemann, 1994). Briefly, V_t was measured with calomel electrodes connected to the chamber via agar bridges made with solution 1. Transepithelial current pulses were passed via Ag/AgCl wires. Sample-and-hold circuitry was used to obtain a signal proportional to R_t from the voltage response to the current pulses (50 nA for 34 msec at 0.3 Hz). V_t and R_t were recorded on a 2-pen chart recorder. Representative traces were digitized omitting, for clarity, the responses to the current pulses (Figs. 4 and 5). The equivalent short-circuit current (I_{sc}) was obtained according to Ohm's law from measurements of V_t and R_t ($I_{sc} = V_t/R_t$). I_{sc} and R_t were normalized for the area defined by the aperture of the micro-Ussing chamber (diameter of aperture: 80 μm).

Determination of pH-Sensitivities

The sensitivities of V_t, R_t and I_{sc} to pH_i and to the extracellular pH (pH_o) were obtained as slopes from linear regressions (Figs. 6 and 7).

For the determination of the pH_i-sensitivity of V_t, ΔV_t during the initial acidification was corrected (reduced by 1.7 mV) for a transient voltage deflection due to the basolateral Cl^- concentration from 153 to 133 mM during the addition of propionate$^-$. The V_t transient due to a Cl^- step from 153 to 133 mM was estimated to be 1.7 mV according to a transference number of 0.46 obtained from the experiments involving Cl^- concentration steps from 153 to 15 mM which resulted in a V_t transient of 28 mV (Wangemann, 1995).

Materials

2′,7′-biscarboxyethyl-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM) was purchased from Molecular Probes (Eugene, OR). Nigericin was obtained from Sigma (St. Louis, MO) and all other chemicals were either purchased from Fluka (Ronkonkoma, NY) or Sigma.

Solutions

The compositions of solutions are listed in Table 1. BCECF-AM was predissolved in DMSO to a final concentration of 0.1%. Nigericin was predissolved in ethanol to a final concentration of 0.1%.

Statistics

Data are presented as arithmetic means ± SEM. The number of observations (n) is the number of tissues. Statistical analysis was performed using the Student's t-test for paired samples. Differences were assumed to be significant when $P < 0.05$.

Results

Under control conditions (solution 1, on both sides of the epithelium), pH_i was 7.01 ± 0.04 (n = 18) and, as reported earlier (Marcus et al., 1994), V_t, R_t and I_{sc} were 9.1 ± 0.5 mV, 16.7 ± 0.9 Ωcm^2 and 587 ± 30 μA/cm^2 (n = 49), respectively.

Effect of Propionate$^-$ on pH_i

Changes in pH_i were induced by addition and removal of 20 mM propionate$^-$ (solution 2). Addition of propionate$^-$ to both the apical and basolateral perfusate caused an initial acidification from pH 6.94 ± 0.04 to pH 6.81 ± 0.03 (*a–b*, Fig. 3) and a subsequent alkalinization to pH 7.14 ± 0.05 (n = 13; *b–c*, Fig. 3). Removal of propionate caused a transient alkalinization to a peak of pH 7.51 ± 0.07 (n = 13; *c–d*, Fig. 3) after which pH_i returned to control values.

Effect of Propionate$^-$ on V_t, R_t and I_{sc}

V_t across vestibular dark cell epithelium is generated by the electromotive force (EMF) associated with the K^+ conductance in the apical membrane and the EMF associated with the Cl^- conductance in the basolateral membrane. If any conductive pathway would be pH sensitive, it would be expected that propionate$^-$ has an effect on V_t and I_{sc}. Addition of propionate$^-$ to the apical or basolateral perfusate caused a biphasic response of V_t, R_t and I_{sc}. This biphasic response involved an initial in-

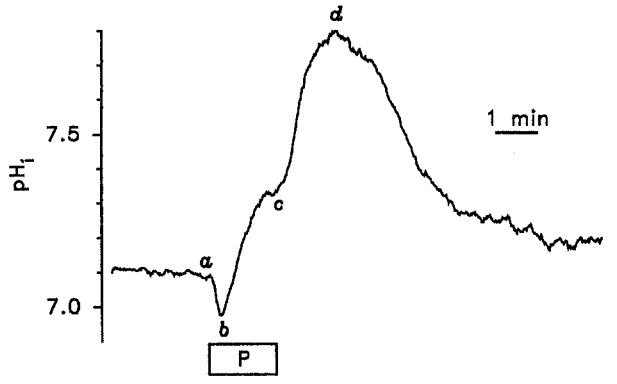

Fig. 3. Effect of 20 mM propionate$^-$ (*P*) on the cytosolic pH (pH_i) of vestibular dark cell epithelium. Compare *a-d* to Figs. 4 and 5.

crease of V_t and I_{sc} and decrease of R_t (*a-b* or *a'*-b', Fig. 4, Table 2) and a subsequent decrease of V_t and I_{sc} and increase of R_t (*b–c* or *b'–c'*, Fig. 4 and Table 2). Removal of propionate$^-$ caused a transient decrease in V_t and I_{sc} and a transient increase in R_t (*c–d* or *c'–d'*, Fig. 4, Table 2). Propionate$^-$ exerted a significantly larger effect from the basolateral side than from the apical side (Fig. 4, Table 2).

Effect of the extracellular pH (pH_o) on V_t, R_t and I_{sc}

Reduction of pH_o of the basolateral perfusate from pH 7.4 to either pH 7.1, pH 6.8 or pH 6.4 (solutions 3–6) caused a biphasic response of V_t, R_t and I_{sc}. This biphasic response involved an initial increase of V_t and I_{sc} and decrease of R_t (a'–b', Fig. 5, Table 3) and a subsequent decrease of V_t and I_{sc} and increase of R_t (b'–c', Fig. 5, Table 3). Return to control pH caused a transient decrease in V_t and I_{sc} and a transient increase in R_t (*c'–d'*, Fig. 5, Table 3). A reduction of pH_o of the apical perfusate from 7.4 to 6.4 caused a monophasic increase in V_t and I_{sc} but had no significant effect on R_t (*a–b*, Fig. 5, Table 3).

Discussion

Propionate$^-$ has been used in a variety of preparations as a tool to induce a cytosolic acidification (Cala & Maldonado, 1994; Rowe, Lesho & Montrose, 1994, Wangemann & Shiga, 1994). As a first step, propionic acid enters the cell by nonionic diffusion since a propionate$^-$ containing solution at pH 7.4 contains 0.3% undissociated propionic acid (pK_a 4.87) which is highly lipid soluble and equilibrates readily across cell membranes (Walter & Gutknecht, 1984). At least in some epithelial cells such as kidney proximal tubule, additional propionic acid uptake occurs via a H^+/monocarboxylate$^-$ cotransport (Siebens & Boron, 1987; Nakhoul & Boron, 1988). As a second step, cytosolic propionic acid dissociates into propionate$^-$ and H^+ which causes the observed cytosolic acidification (*a–b*, Fig. 3). Removal of propionate$^-$ from the perfusate has the reverse effect. Cytosolic propionate$^-$ associates with H^+ and forms the lipid soluble propionic acid which leaves the cell via nonionic diffusion, thereby causing the observed alkalinization of the cytosol (*c–d*, Fig. 3).

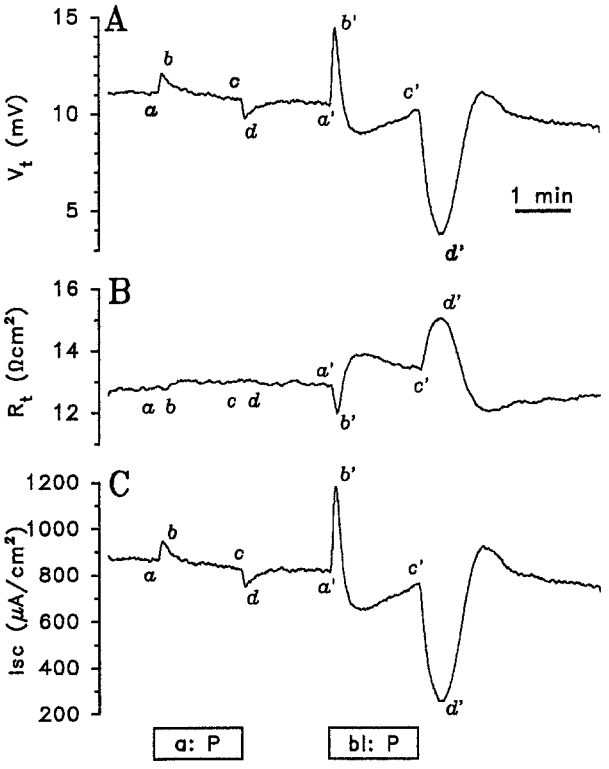

Fig. 4. Effect of apical (a:) and basolateral (bl:) 20 mM propionate$^-$ (*P*) on *A*, the transepithelial voltage (V_t) *B*, the transepithelial resistance (R_t) and *C*, the equivalent short circuit current (I_{sc}). Compare *a-d* to Figs. 3 and 5.

The magnitude of the propionate$^-$-induced cytosolic acidification depends mostly on the presence or absence of acid extrusion mechanisms or base uptake mechanism and on the cytosolic buffer capacity. For example, the propionate$^-$-induced cytosolic acidification was significantly larger in vestibular transitional cells than reported here in vestibular dark cells (Wangemann & Shiga, 1994). The observation that the propionate$^-$-induced acidification was only transient suggests that vestibular dark cells contain an acid extrusion and/or a base uptake mechanism. Indeed, pharmacological evidence suggests that vestibular dark cells contain for the extrusion of acid a basolateral Na^+/H^+ exchanger (P. Wangemann, J. Liu and N. Shiga, *submitted*). In addition, they contain an uptake mechanism for the base propionate$^-$ which might be similar to the Na^+ or Cl^- coupled monocarboxylate transporters described in kidney proximal tubules (Siebens & Boron, 1987; Nakhoul & Boron, 1988; Schild, Aronson & Giebisch, 1990). Uptake of propionate$^-$ results in an alkalinization of the cytosol since cytosolic propionate$^-$ associates with cytosolic H^+ and forms the lipid soluble propionic acid which leaves the cell via nonionic diffusion. Evidence for the presence of a pro-

Table 2. Data summary of the effects of apical and basolateral propionate$^-$ on the transepithelial voltage (V_t), transepithelial resistance (R_t) and the equivalent short-circuit current (I_{sc})

Apical	Control	Propionate$^-$		Control	n
	a	b	c	d	
V_t (mV)	8.0 ± 1.4	9.7 ± 1.4*	7.3 ± 1.5*	6.0 ± 1.5*	2
R_t (Ωcm^2)	12.7 ± 2.0	12.4 ± 1.9^{ns}	12.0 ± 1.5^{ns}	12.1 ± 1.5^{ns}	6
I_{sc} ($\mu A/cm^2$)	636 ± 86	790 ± 75*	591 ± 93*	484 ± 96*	6
Basolateral	Control	Propionate$^-$		Control	n
	a′	b′	c′	d	
V_t (mV)	10.5 ± 0.7	15.9 ± 0.9*	8.8 ± 0.6*	3.0 ± 0.3*	27
R_t (Ωcm^2)	17.1 ± 1.2	15.4 ± 1.1*	18.5 ± 1.4*	20.0 ± 1.6*	27
I_{sc} ($\mu A/cm^2$)	653 ± 39	1107 ± 61*	505 ± 32*	156 ± 13*	27

Compare a–d and a′–d′ to original recordings shown in Fig. 4 and to measurements of pH_i shown in Fig. 3. Significant (*) and insignificant (^{ns}) changes are labeled. The number of experiments (n) is given.

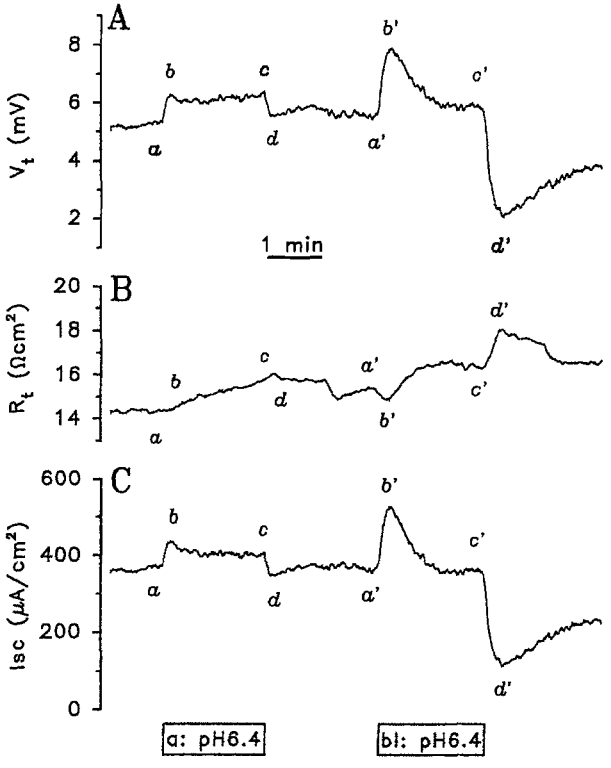

Fig. 5. Effect of apical (a:) and basolateral (bl:) changes in the extracellular pH (pH_o) on *A*, the transepithelial voltage (V_t) *B*, the transepithelial resistance (R_t) and *C*, the equivalent short circuit current (I_{sc}).

pionate$^-$ uptake mechanism comes from the observation, that pH_i in the presence of propionate$^-$ exceeded pH_i under control conditions (*a vs. c*, Fig. 3). Similar observations have been made in kidney proximal tubules (Siebens & Boron, 1987; Nakhoul & Boron, 1988). An alkalinization where pH_i exceeds pH_i under control conditions cannot be explained by a Na^+/H^+ exchanger since cytosolic alkalinization is known to down regulate the Na^+/H^+ exchanger (Aronson, 1985; Grinstein et al., 1985; Wangemann, Shign & Marcus, 1993).

The rate of K^+ secretions across vestibular dark cells can be estimated from the I_{sc} to be about 6 nmol/(s · cm^2) since the I_{sc} (587 $\mu A/cm^2$) is related via the Faraday constant (9.45×10^4 *A* · s/mol) to the transepithelial K^+ secretion (Marcus & Marcus, 1987). Vestibular dark cells take up K^+ across the basolateral membrane via the $Na^+/Cl^-/K^+$ cotransporter and the Na,K-ATPase (Wangemann & Marcus, 1990; Marcus et al., 1994). Cl^- taken up via the $Na^+/Cl^-/K^+$ cotransporter recirculates mostly via a 95 pS Cl^- channel (Marcus et al., 1993) and K^+ is released across the apical membrane via the slowly activating K^+ (I_{sK}) channel (Marcus & Shen, 1994). Due to the electromotive forces associated with the K^+ conductance in the apical membrane and the Cl^- conductance in the basolateral membrane, vestibular dark cells generate a positive V_t when bathed in symmetrical NaCl solutions (Marcus et al., 1994).

A pH-dependency of V_t, R_t or I_{sc} would be expected if the K^+ and/or the Cl^- conductance would be pH-sensitive or if any ion transport mechanism which is involved in maintaining the cytosolic K^+ and Cl^- concentrations would be pH-sensitive. The observation that the time course of V_t was nearly a mirror image of the time course of pH_i and that the time course of R_t was parallel to that of pH_i suggests that the effects on V_t, R_t and I_{sc} were correlated to the changes in pH_i (Figs. 3 and 4). Small deviations from these correlations occurred during and after basolateral propionate$^-$ steps (Fig. 4). During basolateral propionate$^-$ steps V_t and I_{sc} increased slowly whereas R_t decreased slowly (between *b′* and *c′*). Further, after the removal of basolateral propionate$^-$ a small and transient increase in V_t and I_{sc} and decrease in R_t was observed (after d′, Fig. 4). The pH_i-sensitivities of V_t, R_t and I_{sc} between pH 6.8 and pH 7.5 were −15 mV/pH-unit ($r = 0.99$), 6.2 Ωcm^2/pH-unit ($r = 0.97$) and −1089 $\mu A/cm^2$ · pH-unit ($r = 0.97$; Fig. 6). The pH_i-sensitivity of transepithelial K^+ secretion can be estimated to be −12 nmol/(s · cm^2 · pH-unit). Linearity of this correlation exceeding the tested range, however, is unlikely. On the one side, linear extrapolation leads to a cessation of transepithelial K^+ secretion at about pH_i 7.6.

Table 3. Data summary of the effects of changes in the pH of the apical and basolateral perfusate on the transepithelial voltage (V_t), transepithelial resistance (R_t) and the equivalent short circuit current (I_{sc})

Apical	pH 7.4	pH 6.4	pH 6.4	pH 7.4	n
	a	b	c	d	
V_t (mV)	7.3 ± 0.68	8.0 ± 0.7*	7.3 ± 0.5*	6.5 ± 0.5*	9
R_t (Ωcm^2)	15.1 ± 1.0	15.0 ± 1.0^{ns}	15.0 ± 1.1^{ns}	15.2 ± 1.1^{ns}	9
I_{sc} ($\mu A/cm^2$)	494 ± 47	546 ± 50*	508 ± 48*	446 ± 45*	9
Basolateral	pH 7.4	pH 6.4	pH 6.4	pH 7.4	n
	a′	b′	c′	d′	
V_t (mV)	6.6 ± 0.5	8.9 ± 0.7*	6.6 ± 0.6*	3.4 ± 0.4*	14
R_t (Ωcm^2)	16.3 ± 2.1	16.2 ± 2.2^{ns}	17.5 ± 2.2*	18.7 ± 2.3*	14
I_{sc} ($\mu A/cm^2$)	460 ± 44	621 ± 59*	428 ± 45*	222 ± 36*	14
Basolateral	pH 7.4	pH 6.8	pH 6.8	pH 7.4	n
	a′	b′	c′	d	
V_t (mV)	7.7 ± 0.8	9.2 ± 0.8*	7.6 ± 1.0*	5.4 ± 0.9*	6
R_t (Ωcm^2)	22.4 ± 2.9	21.7 ± 2.9*	23.1 ± 3.4^{ns}	24.4 ± 3.9^{ns}	6
I_{sc} ($\mu A/cm^2$)	374 ± 69	466 ± 83*	364 ± 73*	250 ± 58*	6
Basolateral	pH 7.4	pH 7.1	pH 7.1	pH 7.4	n
	a′	b′	c′	d	
V_t (mV)	8.4 ± 1.0	9.3 ± 1.1*	8.4 ± 1.1*	7.2 ± 0.9*	6
R_t (Ωcm^2)	19.0 ± 2.4	18.6 ± 2.5*	19.5 ± 3.0^{ns}	20.1 ± 3.1^{ns}	6
I_{sc} ($\mu A/cm^2$)	467 ± 73	529 ± 81*	465 ± 82*	386 ± 67*	6

Compare a–d and a′–d′ to original recordings shown in Fig. 5. Significant (*) and insignificant (^{ns}) changes are labeled. The number of experiments (n) is given.

On the other side, elevation of transepithelial K^+ secretion might be limited by the maximal transport rates of the mechanisms involved in basolateral K^+ uptake and apical K^+ release. Basolateral K^+ uptake is most likely rate limiting considering that ion channels in general saturate at higher transport rates than cotransporters or ATPases such as the $Na^+/Cl^-/K^+$ cotransporter and the Na,K-ATPase.

The observation that cytosolic acidification caused an increase in V_t and I_{sc} and a decrease of R_t cannot be explained with a primary pH-effect on the basolateral Cl^- conductance. Opening of the Cl^- conductance would decrease R_t as observed (*a′–b′*, Fig. 4), however, the membrane potential of vestibular dark cells was found in preliminary experiments to hyperpolarize during the cytosolic acidification induced by the addition of propionate$^-$ (*unpublished results*). If the primary effect of the cytosolic acidification was due to the basolateral Cl^- conductance, a depolarization rather than a hyperpolarization of the membrane potential would have been expected. Further, a primary effect on the Cl^- conductance is unlikely since the 95 pS Cl^- channel which is the major component of the basolateral Cl^- conductance has been found to be insensitive to pH-changes (Marcus et al., 1993). The observed effects on V_t, R_t and V_c are consistent, however, with the hypothesis that acidification caused an increase in the apical K^+ conductance. Two K^+ conductive pathways have recently been found in the apical membrane of vestibular dark cells, the I_{sK} channel which is the major K^+ conductive pathway and responsible for K^+ secretion (Marcus & Shen, 1994) and the maxi-K^+ channel which might not play a significant role under unstimulated conditions (Takeuchi, Marcus & Wangemann, 1992). A direct stimulatory effect of acidification on the maxi-K^+ channel is unlikely since the maxi-K^+ channel has been found in a variety of preparations to be inhibited by cytosolic acidification (Stampe & Vestergaard Bogind, 1985; Copello, Segal & Reuss, 1991). Also most other K^+ channels are known to be inhibited by a cytosolic acidification (Ohno-Shosaku et al., 1990; Fan, Tokuyama & Makielski, 1994; Schlatter et al., 1994). In contrast, cytosolic acidification in vestibular dark cells caused most likely a transient stimulation of the apical I_{sK} channel. Whether this stimulation occurred directly or indirectly, i.e., via a mediator, remains unknown, since the pH-sensitivity of the I_{sK} channel has not yet been studied in a cell-free system where the distinction could be made between a direct and an indirect pH effect.

A mediator between pH_i and the I_{sK} channel could conceivably involve the cytosolic Ca^{2+} concentration but less likely the membrane potential. The cytosolic Ca^{2+} concentration as a mediator between the cytosolic acidification and the activation of the I_{sK} channel is conceivable since it has been shown in preparations other than vestibular dark cells that an extracellular or cytosolic acidification causes an increase in the cytosolic Ca^{2+} concentration (Sato, 1994) and that an elevated cytosolic

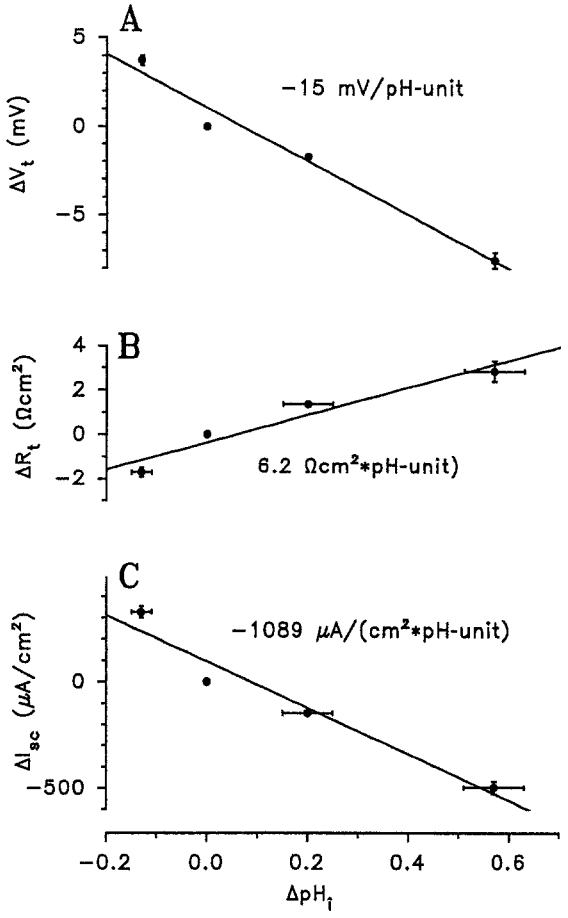

Fig. 6. Sensitivity of *A* the transepithelial voltage (V_t), *B* the transepithelial resistance (R_t) and *C* the equivalent short circuit current (I_{sc}) to changes in the cytosolic pH (pH_i) induced by 20 mM propionate$^-$ applied to the basolateral perfusate. The values for pH_i were obtained from experiments as shown in Fig. 3 and the values for V_t, R_t and I_{sc} were obtained from experiments as shown in Fig. 4. The pH_i-sensitivities were obtained as slopes of the linear regressions between changes in pH_i and changes in V_t, R_t and I_{sc}.

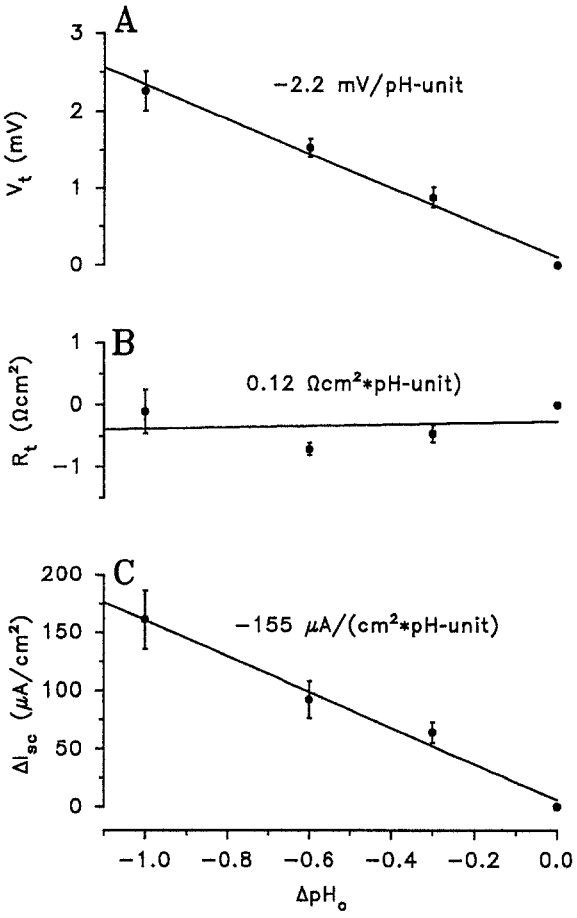

Fig. 7. Sensitivity of *A* the transepithelial voltage (V_t), *B* the transepithelial resistance (R_t) and *C* the equivalent short circuit current (I_{sc}) to changes in the extracellular pH (pH_o) of the basolateral perfusate. The values for V_t, R_t and I_{sc} were obtained from experiments as shown in Fig. 5. The pH_o-sensitivities were obtained as slopes of the linear regressions between changes in pH_o and changes in V_t, R_t and I_{sc}.

Ca^{2+} concentration possibly via a calmodulin-dependent protein kinase activates the I_{sK} channel (Honore et al., 1992). The membrane voltage, however, is an unlikely mediator between acidification and activation of the I_{sK} channel since cytosolic acidification coincided with a hyperpolarization of the membrane potential and since hyperpolarizations are known to inactivate the I_{sK} channel (Marcus & Shen, 1994).

Acidification of the basolateral perfusate most likely caused at least a transient acidification of pH_i. This acidification could be mediated via the basolateral Na^+/H^+ exchanger due to the reduction in the proton gradient in the presence of continuous metabolic acid production. The observed initial increase in V_t and I_{sc} and decrease in R_t were most likely due the pH_i-sensitivity of the apical membrane, i.e., the I_{sK} channel. Support for the hypothesis comes from the observation that acidification of the apical perfusate caused a similar but faster effect on V_t and I_{sc} (Fig. 5). The reason for the observation that the response was larger when the change in pH_o was applied to the basolateral side might be related to the fact that the area of the basolateral membrane is about 40 times larger than the apical membrane area (W. ten Cate, *personal communication*). Similar reasoning applies also to the observation that propionate$^-$ caused a larger effect on V_t and I_{sc} when applied basolaterally than when applied apically.

The pH_o-sensitivites of V_t, R_t and I_{sc} obtained from the initial responses (a′–b′, Fig. 5, Table 3) to basolateral pH_o steps between pH 7.4 and pH 6.4 were −2.2 mV/pH-unit (r = 0.99), −0.2 Ωcm^2/pH-unit (r = 0.12) and −155 mA/cm^2 · pH-unit (r = 0.99; Fig. 7) and the pH_o-sensitivity of transepithelial K^+ secretion was estimated to be −1.6 nmol/(s · cm^2 · pH-unit). Comparison of the pH_i- and the pH_o-sensitivity of I_{sc} suggests that an extracellular acidification caused a cytosolic acidification which was smaller by about a factor of 7, thus a change in pH_o e.g., from 7.4 to 7.2 would cause a change in pH_i from 7.00 to 6.97.

The observed decline in V_t and I_{sc} and increase in R_t observed during the extracellular acidification (b'–c', Fig. 5, Table 3) cannot be explained by a change in pH_i since pH_i most likely declined monophasically rather than transiently in the presence of an extracellular acidification. The observed secondary phase was most likely related to some kind of cross-talk between the apical I_{sK} channel and the K^+ uptake mechanisms in the basolateral membrane. Indeed, the Na,K-ATPase which is part of the basolateral K^+ uptake mechanism has been shown to be inhibited during a cytosolic acidification (Kuijpers & Bonting, 1969) and inhibition of the $Na^+/Cl^-/K^+$ cotransporter which is the other basolateral K^+ uptake mechanism has been shown to cause via cross-talk inhibition of the I_{sK} channel (Marcus & Shen, 1994). Accordingly, cytosolic acidification would initially result in a stimulation of the I_{sK} channel which would be subsequently overridden by the inhibitory cross-talk effect.

In conclusion, the present data demonstrate that transepithelial K^+ transport across vestibular dark cell epithelium is sensitive to pH_i and pH_o and suggest that cytosolic acidification activates and that cytosolic alkalinization inactivates the I_{sK} channel in the apical membrane. Whether the effect of pH_i on the I_{sK} channel is a direct or indirect effect remains to be demonstrated.

The authors wish to thank Drs. Daniel C. Marcus, Zhjiun Shen and Hiroshi Sunose for helpful discussions. This work was supported by grants NIH-R29-DC01098 and NIH-R01-DC00212.

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