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Implications of an Anomalous Intracellular Electrical Response in Bullfrog Corneal Epithelium

Peter Reinach and Wolfram Nagel

Washington University School of Medicine, Departments of Ophthalmology and Physiology & Biophysics, St. Louis, Missouri 63110 and Physiologisches Institut, Universitat Munchen, Munich, West Germany

Summary. The ionic dependencies of the transepithelial and intracellular electrical parameters were measured in the isolated frog cornea. In NaCl Ringer's the intracellular potential difference V_{sc} measured under short-circuit conditions depolarized by nearly the same amount after either increasing the stromal-side KCl concentration from 2.5 to 25 mM or exposure to 2 mM $BaCl_2$ (K^+ channel blocker). With Ba^{2+} the depolarization of the V_{sc} by 25 mM K^+ was reduced to one-quarter of the control change. If the Cl-permselective apical membrane resistance R_o remained unchanged, the relative basolateral membrane resistance R_i, which includes the lateral intercellular space, increased at the most by less than twofold after Ba^{2+}. These effects in conjunction with the depolarization of the V_{sc} by 62 mV after increasing the stromal-side K^+ from 2.5 to 100 mM in Cl-free Ringer's as well as the increase of the apparent ratio of membrane resistances ($a = R_o/R_i$) from 13 to 32 are all indicative of an appreciable basolateral membrane K^+ conductance. This ratio decreased significantly after exposure to either 25 mM K^+ or Ba^{2+}. The decline of R_o/R_i with 25 mM K^+ appears to be anomalous since this decrease is not consistent with just an increase of basolateral membrane conductance by 25 mM K^+, but rather perhaps a larger decrease of R_o than R_i. Also an increase of lateral space resistance may offset the effect of decreasing R_i with 25 mM K^+. In contrast, R_o/R_i did transiently increase during voltage clamping of the apical membrane potential difference V_o and exposure to 25 mM K^+ on the stromal side. This increase and subsequent decrease of R_o/R_i supports the idea that increases in stromal K^+ concentration may produce secondary membrane resistance changes. These effects on R_o/R_i show that the presence of asymmetric ionic conductance properties in the apical and basolateral membranes can limit the interpretative value of this parameter. The complete substitution of Na^+ with *n*-methyl-glucamine in Cl-free Ringer's on the stromal side hyperpolarized the V_{sc} by 6 mV whereas 10^{-4} M ouabain depolarized the V_{sc} by 7 mV. Thus the basolateral membrane contains K^+, Na^+ and perhaps Cl^- pathways in parallel with the Na/K pump component.

Key Words corneal epithelium · frog · chloride transport · potassium conductance · apical and basolateral membranes · barium · ouabain

Introduction

Cl translocation across the frog corneal epithelium includes carrier-mediated Na/Cl uptake at the basolateral membrane followed by electrodiffusion across the essentially Cl-selective apical membrane into the tear-side bathing solution (Reuss et al., 1983). At constant intracellular Cl^- activity the magnitude of Cl^- efflux across the apical membrane is determined by the negativity of the intracellular potential difference. The origin of this negativity has not been characterized in the frog corneal epithelium. Previously, Reuss et al. (1983) found intracellular K^+ activity to be significantly above electrochemical equilibrium. This result suggests that the negativity of the intracellular potential difference in the frog corneal epithelium may be due to an appreciable K^+ conductance in the basolateral membrane as proposed for other epithelia. Support for this contention is based in part on the effects of a K^+ channel blocker, Ba^{2+}, on the electrical parameters of a variety of epithelia (Nagel, 1979; Nielsen, 1979; Kirk et al., 1980; McLennan et al., 1980; Van Driessche & Zeiske, 1980; Biagi et al., 1981; Planelles et al., 1981; Reuss et al., 1981; Bello-Reuss, 1982; Wills et al., 1982; Greger & Schlatter, 1983; Kirk & Dawson, 1983; Koeppen et al., 1983; Welsh, 1983; Candia et al., 1984; O'Neil & Sansom, 1984).

In this study, we characterize the ionic dependency of the basolateral membrane conductance by measuring the effects of single ion substitutions of either Na^+, K^+ or Cl^- in the stromal bathing solution on the transepithelial and intracellular electrical parameters. In addition, the effects of Ba^{2+} and ouabain were considered. The results indicate that the K^+ conductance of the basolateral membrane is appreciable relative to a small Na^+ conductance. The electrical driving force for Cl^- efflux across the apical membrane results in part from the large K^+ concentration gradient partitioned between the cell interior and the stromal bathing solution by a K^+-conductive basolateral membrane.

Materials and Methods

Corneas of the bullfrog, *Rana catesbeiana*, were isolated from doubly pithed animals, mounted in a special flow-through Uss-

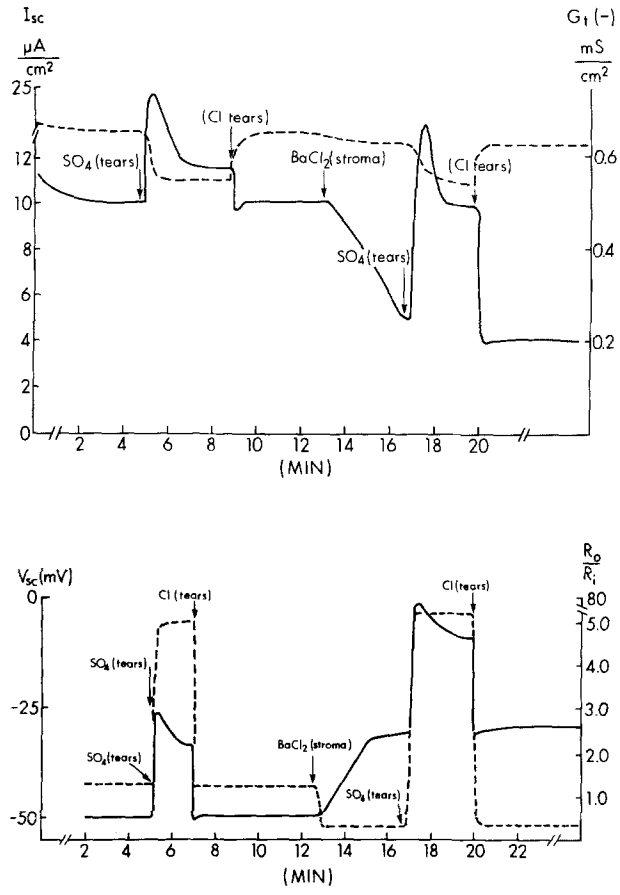

Fig. 1. *Upper:* Effects of $BaCl_2$ on transepithelial electrical parameters. Bullfrog corneas were bathed in NaCl Ringer's and short circuited (V_t = 0). Solid and dashed lines represent the short-circuit current I_{sc} and transepithelial electrical conductance g_t, respectively. NaCl substitution with Cl-free Ringer's (tears) is a validation technique of intracellular impalement. $BaCl_2$ (2 mM) superfusion was done on stromal side. *Lower:* Effects of $BaCl_2$ on intracellular electrical parameters. Intracellular electrical potential difference under short-circuit conditions V_{sc} is shown with the solid line. The dashed lines represent the apparent ratio of membrane resistances (R_o/R_i) NaCl substitution with Cl-free Ringer's on the tear side results in significant V_{sc} depolarization and increase of R_o/R_i in adequately impaled cells. $BaCl_2$ was added to the stromal solution and after restabilization of the parameters the effects of NaCl substitution with Cl-free Ringer's on V_{sc} and R_o/R_i indicate adequate cellular impalement

ing-type chamber with 0.3 ml volume on either side of the tissue (Nagel, 1978) and continuously perfused on both sides (flow rate 2.5 ml/min) with either NaCl or Na_2SO_4 Ringer's solution. The composition of the NaCl Ringer's solution was (in mM): Na^+ 110, K^+ 2.5, Ca^{2+} 1, Cl^- 113, glucose 5, HEPES 3.5; pH 8.1; osmolality 220 mOsm. Na_2SO_4 Ringer's (i.e. Cl-free) contained (in mM): Na_2SO_4 55, K_2SO_4 1.25, Ca^{2+} 1, glucose 5, HEPES 3.5; pH 8.1; osmolality adjusted with sucrose to 220 mOsm. KCl or K_2SO_4 was substituted for NaCl or Na_2SO_4 on an equimolar basis to yield K^+ concentrations of 5, 7.5 or 25 mM (and 56 and 100 mM in Cl-free solution). Cl-free Ringer's which was Na-free contained 110 mM *n*-methyl-D-glucamine sulfate (NMDG).

Transepithelial potential difference (V_t) was measured with flowing 3 M KCl bridges connected to calomel electrodes. The bridge endings were 0.5 mm from the tissue surface assuring constant series resistance in the bathing solutions of less than 10 Ω cm². Circular Ag/AgCl electrodes 4 mm from each surface of the tissue served to pass transepithelial current. The corneas were voltage clamped by means of an automatic clamping device (Frankenberger & Nagel, *in preparation*); unless otherwise stated, they were maintained at V_t = 0 mV, i.e. short-circuited. Periodic perturbation of V_t by 10 mV for 100 to 250 msec at rates of 0.5 to 1 Hz was used to measure the transepithelial electrical conductance g_t from the induced change in I_t. The pulse duration was long enough to dissipate capacitative transients as observed on an oscilloscope.

The intracellular potential difference V_{sc} (tear: reference), was recorded with microelectrodes prepared from Omega-dot tubing (F. Haer, Ann Arbor, Mich.) using a Brown Flaming P-77 puller and backfilled with 1.5 M KCl. These electrodes, with input resistance between 30 and 80 MΩ and tip potentials below 5 mV, were connected via a AgCl-coated Ag wire to a high input impedance preamplifier (Analog Devices 515J) with negative capacitance compensation. Impalements of the corneal epithelium were done as previously described (Nagel & Reinach, 1980) from the tear-side perpendicular to the surface using a stepping motor micromanipulator (Frankenberger, Germering, Munich, FRG). The above-mentioned periodic perturbations of V_t were also used to measure the fractional resistance of the apical membrane fR_o defined as the resistance of the apical membrane divided by the transcellular electrical resistance R_c: $fR_o = \Delta V_o/\Delta V_t = R_o/R_c = R_o/(R_o + R_i)$. R_i is a lumped parameter which includes the resistance of both the basolateral membrane and the lateral interstitial pathway. Therefore, fR_o is an apparent ratio of the relative apical membrane resistance. With good intracellular impalements, the scatter of fR_o was typically below ±1%. Similarly, the apparent ratio of membrane resistances ($a = R_o/R_i$) was calculated from the relationship: $a = -fR_o/(fR_o - 1)$. In most cases, the microelectrode input resistance was measured at the rate of 0.5 to 1 Hz. In addition to the usual criteria used to validate a particular impalement, we used the observation that the substitution of NaCl with Cl-free Ringer's in the tear-side bathing solution resulted in a reversible and significant depolarization of V_{sc} as well as a substantial increase of fR_o, resulting from the fact that the apical membrane is essentially Cl-selective (Reuss et al., 1983). It is understood that this criterion is not as rigid as for example using amilioride in Na-transporting epithelia. Nevertheless, it allows exclusion of grossly inadequate impalements.

The values of V_t, I_t, g_t, V_o and fR_o were continuously recorded on a multi-channel strip chart recorder (BBC-Metrawatt 460) which has a response time of 300 msec for full scale deflection. All values are reported as means ± SEM. Statistical analysis was performed using paired Students *t*-test.

Results

Effects of Ba^{2+} on the Stromal Side

In Fig. 1 (upper panel) are shown the effects of stromal-side superfusion with 2 mM $BaCl_2$ on the transepithelial electrical parameters. Barium addition caused the I_{sc} to decline by 64% in less than 10 min after an onset delay of approximately 1 min.

Table 1. Effects of 2 mM $BaCl_2$ on transepithelial and intracellular electrical parameters of frog corneas in NaCl Ringer's (n = 14)

a. Transepithelial	I_{sc} (μA/cm^2)	g_t mS/cm^2
Control	12.9 ± 1.6	1.13 ± 0.16
$BaCl_2$	5.0 ± 1.1	1.02 ± 0.16
b. Intracellular	V_{sc} (mV)	R_o/R_i
Control	−53 ± 5	0.89 ± 0.06
$BaCl_2$	−36 ± 3	0.41 ± 0.4[a]

[a] $P < 0.001$.

The transepithelial conductance decreased by 9%. The average effects of 2 mM $BaCl_2$ on the transepithelial parameters are summarized in Table 1*a*, indicating that the I_{sc} and g_t decreased by 61 and 10%, respectively. The effects of Ba^{2+} were partially reversible in eight experiments where the I_{sc} was initially inhibited by 60% with 2 mM Ba^{2+}. Thirty minutes after the removal of Ba^{2+} the I_{sc} recovered to a value that was 35% smaller than the control value.

The responses of the intracellular electrical parameters after Ba^{2+} are shown in Fig. 1 (lower panel). The control value of V_{sc} was −49 mV and R_o/R_i was 1.0. Within 3 min after exposure to barium the V_{sc} and R_o/R_i decreased to −36 mV and 0.38, respectively. The time intervals for altering the intracellular electrical parameters were always shorter than for the transepithelial electrical parameters. Tear-side superfusion with Cl-free Ringer's in the presence of Ba^{2+} resulted in a larger decrease of the V_{sc} and larger increase of R_o/R_i than under control conditions: R_o/R_i reached as high a level as before barium. In Table 1*b* are summarized the effects of 2 mM Ba^{2+} on the intracellular electrical parameters. The V_{sc} and R_o/R_i decreased significantly suggesting an appreciable basolateral membrane K^+ conductance.

Effects of Increasing K^+ on the Stromal Side

The basolateral membrane K^+ conductance was further assessed by measuring the effects of elevating the K^+ concentration in the stromal bathing solution. A typical record is shown in Fig. 2. The I_{sc} decreased by about 22% whereas g_t increased by about 10% (upper panel). The V_{sc} depolarized in this

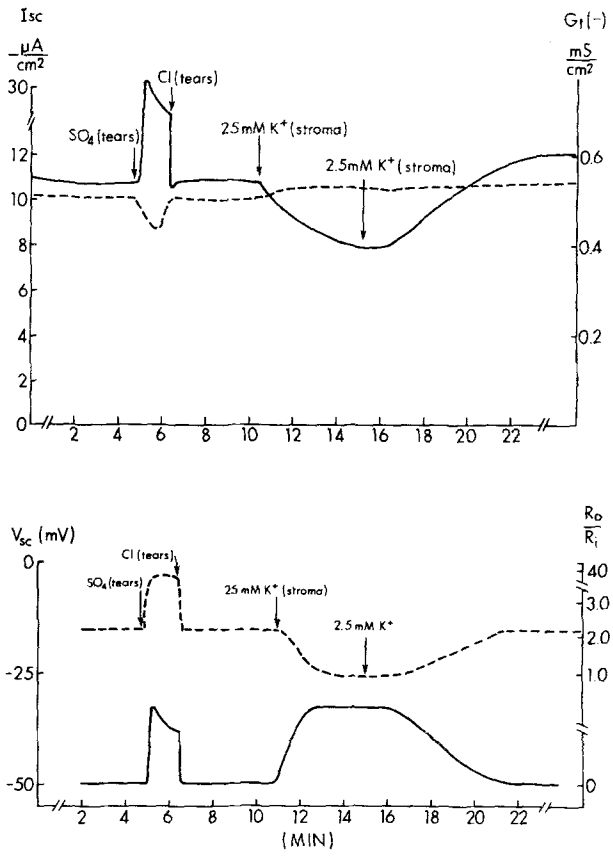

Fig. 2. *Upper:* Effects of 25 mM KCl on transepithelial electrical parameters. In NaCl Ringer's under short-circuiting conditions, the K^+ concentration was increased from 2.5 to 25 mM on the stromal side. Solid and dashed lines represent the short-circuit current and transepithelial electrical conductance, respectively. *Lower:* Effects of 25 mM KCl on intracellular electrical parameters. The solid and dashed lines represent the intracellular potential difference under short-circuit conditions and R_o/R_i, respectively. Validation technique for adequate impalement shows significant depolarization of V_{sc} and increase of R_o/R_i. Superfusion with 25 mM K^+ was done on the stromal side

experiment by 18 mV and R_o/R_i decreased from 2.53 to 1.0 (lower panel). In the presence of 2 mM $BaCl_2$, increasing the K^+ concentration from 2.5 to 25 mM depolarized the V_{sc} in 13 tissues by one-quarter the value observed before the addition of Ba^{2+} (*cf.* Table 2*b*). It should be noted that the I_{sc} decreased much less after 25 mM K^+ than after Ba^{2+} despite comparable depolarization of the V_{sc}. Restoration of 2.5 mM K^+ resulted in the complete reversal of all parameters to their control values which further validates the effects of 25 mM K^+ on the electrical parameters. In Tables 2*a* and *b* are summarized the effects of 25 mM KCl on the stromal side as well as the reversibility of these effects on the transepithelial and intracellular electrical parameters. Noteworthy is the fact that 25 mM K^+ had a significant

inhibitory effect on the I_{sc} which was smaller than that with Ba^{2+} even though the depolarization of the V_{sc} was nearly the same as with Ba^{2+}.

The effects on the electrical parameters of increasing stromal-side K^+ from 2.5 to 100 mM in Cl-free Ringer's are shown in Table 3. In Cl-free Ringer's, the I_{sc} is very small indicating that intraepithelial current looping is very low. Therefore, there is minimal shunting of the V_{sc}. The slopes of the change in V_{sc} between 2.5 and 25 mM and 25 and 100 mM K^+ were 29 and 33 mV, respectively. The latter value approaches a Nernstian prediction of 35 mV. The slope between 2.5 and 25 mM K^+ in NaCl Ringer's was 19 mV, which is shallower than in Cl-free Ringer's (Table 2*b*). Neither of the transepithelial electrical parameters were altered by this procedure. The values for fR_o gradually increased stepwise and there was a significant difference between the values at 100 mM and 2.5 mM. Therefore, the increase of R_o/R_i is suggestive of a decrease in R_i since under this condition R_o is invariant due to the apical membrane's large Cl^- permselectivity. The possibility for any K^+ conductance in the apical membrane in Cl-free Ringer's was also considered in these experiments. After increasing the tear-side K^+ from 2.5 to 25 mM there was no significant change in either of the intracellular electrical parameters (*data not shown*).

Table 2. Effects of 25 mM KCl on transepithelial and intracellular electrical parameters of frog corneas in NaCl Ringer's (n = 13)

a. Transepithelial

	I_{sc} (μA/cm^2)	g_t (mS/cm^2)
Control	8.6 ± 1.1	0.76 ± 0.11
25 K^+	5.4 ± 1.0[a]	0.81 ± 0.11
Control	9.4 ± 1.1	0.83 ± 0.11

b. Intracellular

	V_{sc} (mV)	R_o/R_i
Control	−52 ± 4	1.0 ± 0.06
25 K^+	−33 ± 10	0.72 ± 0.05[a]
Control	−55 ± 5	1.04 ± 0.06

[a] $P < 0.05$.

The significant decline of R_o/R_i in NaCl Ringer's with 25 mM KCl is not consistent with what is expected from the effect of increasing the stromal K^+ concentration: A decrease in R_i without a change of R_o should have increased the value of R_o/R_i. To characterize this anomalous response of R_o/R_i, the effects of increasing stromal-side K^+ to 25 mM on the electrical parameters were measured under three conditions (Table 4):

(1) NaCl Ringer's in both bathing solutions.
(2) NaCl Ringer's and Na_2SO_4 Ringer's: tear and stromal sides, respectively.

Table 3. Effects of increasing stromal side [K] in Cl-free Ringer's on the transepithelial and intracellular electrical parameters (n = 11)

[K]	I_{sc}	g_t	fR_o	R_o/R_i	V_{sc} (mV)
2.5	0.8 ± 0.3	0.13 ± 0.02	0.93 ± 0.002	13	−78 ± 1
25	0.8 ± 0.3	0.14 ± 0.02	0.94 ± 0.002	16	−49 ± 1
56	0.6 ± 0.3	0.16 ± 0.02	0.94 ± 0.002	19	−38 ± 1
100	0.5 ± 0.3	0.16 ± 0.02	0.97 ± 0.002	32[a]	−16 ± 1

[a] $P < 0.05$ (paired data analysis).

Table 4. Effects of tear-side ion composition on response of R_o/R_i to 25 mM K^+ on stromal side

Tear/stroma	2.5 mM K^+				25 mM K^+			
	I_{sc}	g_t	R_o/R_i	V_{sc}	I_{sc}	g_t	R_o/R_i	V_{sc}
1) NaCl/NaCl (9)	12 ± 2	0.90 ± 0.1	1.2 ± 0.2	−54 ± 3	9 ± 1[a]	0.94 ± 0.1	0.8 ± 0.1[a]	−35 ± 2[a]
2) NaCl/Na_2SO_4 (8)	0.5 ± 0.4	0.40 ± 0.05	4.3 ± 2	−67 ± 3	−1.4 ± 0.6[a]	0.41 ± 0.04	2 ± 1[a]	−42 ± 3[a]
3) Na_2SO_4/Na_2SO_4 (11)	0.8 ± 0.3	0.13 ± 0.02	13 ± 4	−80 ± 1	0.8 ± 0.3	0.14 ± 0.02	16 ± 5	−44 ± 1[a]

[a] $P < 0.05$ (paired data analysis).

(3) Na_2SO_4 Ringer's in both bathing solutions.

The effects of 25 mM K^+ were initially measured in condition 1 followed by restoring 2.5 mM K^+ in NaCl Ringer's. In 8 out of 9 corneas, the effects of 25 mM KCl were reversible. The same experiment was repeated under condition 2. Under condition 1, the I_{sc} declined significantly by 25% whereas g_t did not change significantly. The V_{sc} depolarized by 19 mV and R_o/R_i decreased in an anomalous direction. With condition 2, the I_{sc} reversed direction but the g_t was unaltered. The control V_{sc} value was hyperpolarized with respect to condition 1. This effect and the reversal of the I_{sc} result from the inward-directed Cl^- gradient from the tears. The mean depolarization of the V_{sc} with 25 mM K^+ was 25 mV and R_o/R_i decreased significantly in the anomalous direction. Under condition 3, with a different set of corneas, only the V_{sc} changed significantly. The lack of a significant change of R_o/R_i is indicative of the much higher resistance of R_o relative to R_i in Cl-free Ringer's.

Effects of Potential Difference on R_o/R_i Change

The voltage sensitivity of the anomalous R_o/R_i change was considered by comparing the effects on R_o/R_i of increasing stromal K^+ from 2.5 to 25 mM in Cl-free and NaCl Ringer's while maintaining the apical membrane potential difference V_o at its control value (Fig. 3, left and right panels, respectively). Sending sufficient transepithelial current depolarized V_t to a value below 0 mV. The left panel shows that R_o/R_i increased very slightly but that the secondary decrease was missing. In contrast, the right panel (constant V_o) indicates that R_o/R_i transiently increased and thereafter declined only slightly below its control level. After releasing the clamp and returning to short circuit (i.e. $V_t = 0$), V_o and R_o/R_i assumed values similar to those observed during continuous short-circuiting. Table 5 summarizes the data of these experiments. Provided the tear-side bathing solution contained NaCl Ringer's, R_o/R_i always initially increased significantly from its control value, in contrast to the response at short circuit (*see* Table 4) when R_o/R_i always significantly decreased.

To further examine the voltage sensitivity of the anomalous R_o/R_i change, the responses of V_o and R_o/R_i to clamping of V_t to positive and negative values were measured under the same three conditions which were used to characterize the anomalous change of R_o/R_i. In Fig. 4 the top panel shows the V_t clamp protocol used under the three conditions. The middle panel shows the effects on V_o in a typical experiment under condition 1 ($n = 6$). The bottom panel shows the average responses of R_o/R_i to this voltage-clamp regimen under conditions 1 and 2. It is apparent that the magnitudes of the changes of R_o/R_i were smaller under condition 2 than condition 1. This difference can be partly explained by a significant contribution of the voltage-dependent apical membrane Cl^- conductance to the cellular conductance. With NaCl Ringer's on the tear-side, voltage perturbations significantly altered Cl^- entry

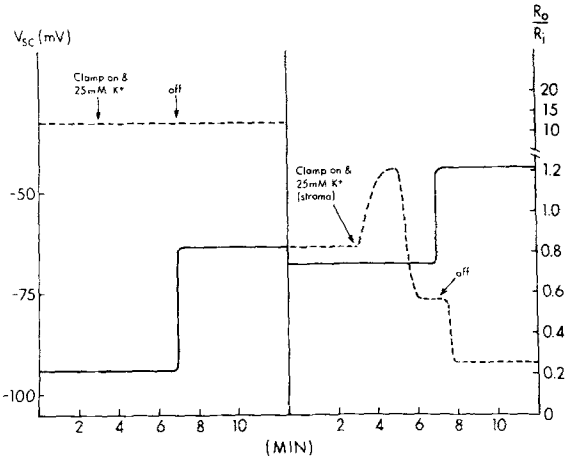

Fig. 3. Effects on R_o/R_i of increasing stromal-side K^+ concentration to 25 mM in Cl-free Ringer's (left) and NaCl Ringer's while voltage clamping V_o at the control value of V_{sc}

Table 5. Effects of voltage clamping V_o on electrical parameters during perfusion with 25 mM K^+ on stromal side

Bath composition tear/stroma	2.5 mM K^+				25 mM K^+			
	I_{sc}	g_t	V_{sc}	R_o/R_i	I_t	g_t	V_o	R_o/R_i
NaCl/NaCl ($n = 6$)	14 ± 3	0.8 ± 0.1	−45 ± 1	0.67 ± 0.15	31 ± 3	0.8 ± 0.1	−45 ± 1	0.84 ± 0.18[b]
NaCl/Na_2SO_4 ($n = 5$)	−1 ± 2	0.61 ± 0.02	−84 ± 1	1.16 ± 0.16	5 ± 2	0.60 ± 0.02	−84 ± 1	1.40 ± 0.16[b]
Na_2SO_4/Na_2SO_4 ($n = 8$)	0.5 ± 0.3	0.47 ± 0.03	−65 ± 3	20 ± 6	11 ± 2	0.47 ± 0.03	−65 ± 3	27 ± 10

[a] Transepithelial current.
[b] $P < 0.05$ (paired data analysis).

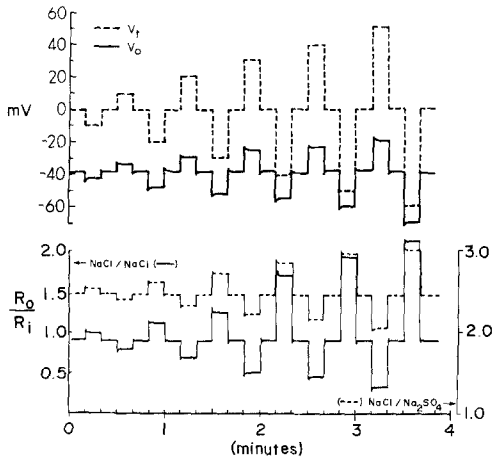

Fig. 4. Effects of alteration of V_t on intracellular electrical parameters. In NaCl Ringer's, sufficient transepithelial electrical current was sent across the frog cornea to vary V_t according to the regimen indicated by the dashed lines. Voltage clamping was maintained at each indicated level for 15 sec. Tissue was short circuited ($V_t = 0$) between each alteration of V_t. Middle panel shows a typical response of V_o in NaCl Ringer's. Bottom panel shows the mean responses of R_o/R_i to voltage clamping in NaCl Ringer's (NaCl/NaCl), left axis ($n = 6$) and with NaCl Ringer's and Na_2SO_4 Ringer's on the tear and stromal sides, respectively (NaCl/Na_2SO_4) ($n = 5$). In Na_2SO_4 Ringer's on both sides R_o/R_i was invariant (*not shown*)

across the apical membrane and perhaps Cl^- efflux across the basolateral membrane. These effects are reflected by appreciable changes of R_o in addition to any changes of R_i. Only under condition 3, was R_o/R_i invariant since any changes at other membrane barriers could not be resolved in the presence of a much higher resistance for R_o.

Effects of Sodium-Free Solution and Ouabain on the Stromal Side

The contribution of basolateral conductances other than K^+ and Cl^- was studied by measuring in Cl-free Ringer's ($I_{sc} \simeq 0$) the effects of either Na^+ removal or incubation with 10^{-4} M ouabain. The complete substitution of *n*-methyl-D-glucamine for Na^+ in six tissues resulted in a significant hyperpolarization of V_{sc} by 6 ± 2 mV and this increase was completely reversed upon restoring Na^+. Neither incubation with $NMDGSO_4$ nor ouabain had any significant effects on either of the transepithelial electrical parameters or R_o/R_i. Ouabain incubation in six tissues resulted in a depolarization of V_{sc} by 7 ± 0.4 mV within 30 sec. In both cases, there were no significant changes of R_o/R_i.

Discussion

There are two reasons preventing quantitation of the basolateral membrane ion permeability:

(1) In order to perform DC circuit analysis, it is necessary to assume that an agent alters the rate of Cl^- transport by only changing the value of a single resistance element in the equivalent circuit. Unlike in electrically high resistance Na^+-transporting epithelia, it is questionable if there is an agent currently available which rapidly and reversibly alters selectively the conductance of a single element in the electrical equivalent of the corneal epithelium. Previously DC circuit analysis was performed based on the effects of either adenosine or epinephrine on the electrical parameters (Nagel & Reinach, 1980; Reuss et al., 1983). The validity of this assumption is time dependent in the studies where adenosine was used. Epinephrine alters the electrical properties of the shunt, the basolateral and apical membranes. Furthermore, we have no knowledge about possible changes in the intracellular ionic activities which may be significant owing to the slow and often poor reversibility of these agents.

(2) It is not possible to restrict the essentially Cl-permselective apical membrane. Despite these limitations a number of important conclusions can be drawn. In Cl-free Ringer's, any changes of the V_{sc} and fR_o associated with increasing the stromal-side K^+ concentration to 100 mM are essentially reflective of alterations in R_i since the apical membrane is far less conductive than the basolateral membrane. Nevertheless, the resolution of any changes of R_i is protocol dependent since R_o/R_i only increased if the stromal-side K^+ was increased to 100 mM (Table 3) or during voltage clamping while superfusing 25 mM K^+ (Fig. 3, left panel). This dependency may be related to the magnitude of depolarization and/or the time duration of current passage.

The depolarization of the basolateral membrane potential difference and the decrease of R_o/R_i by Ba^{2+} are in agreement with the well-documented blocking action of this cation on potassium channels in epithelial membranes (*see* Introduction for references). The magnitude of the change in membrane resistance with Ba^{2+} may be more easily appreciated from a recalculation of the data shown in Table 1*b*. The ratio of the basolateral/apical membrane resistance R_i/R_o shows that R_i/R_o increases from 1.13 to 2.45, which would indicate a twofold increase of R_i if R_o remained unchanged. This is not very likely in view of the voltage sensitivity of the apical membrane conductance (*see below*). This calculated

value should be considered as the upper limit of the increase in basolateral membrane resistance. Assuming that the sensitivity to Ba^{2+} is about the same in the frog cornea as in other tissues, Ba^{2+}-sensitive potassium channels should be nearly completely blocked at 2 mM. Accordingly, the partial conductance of potassium would account for 50% or less of the basolateral membrane conductance in the frog cornea, which is considerably less than other epithelial basolateral membranes: (1) frog skin (Nagel, 1979); (2) canine trachea (Welsh, 1983); (3) rabbit urinary bladder (Lewis et al., 1978); (4) *Necturus* gallbladder (Reuss, 1979).

The effects of increasing stromal-side K^+ to 25 mM on most of the electrical parameters are consistent with the conclusions drawn from the experiments with Ba^{2+}. The depolarization of the epithelial cells indicates a significant basolateral membrane K^+ conductance. Surprisingly, however, R_o/R_i decreased in contrast to what would be expected from constant-field considerations. The experiments summarized in Tables 4 and 5 demonstrate that this anomalous response of R_o/R_i is in part a consequence of an increase of the apical membrane Cl^- conductance since in Cl-free Ringer's R_o/R_i did not change after exposure to 25 mM K^+. However, changing K^+ from 2.5 to 100 mM resulted in a significant (4%) increase in fR_o which corresponds to an increase of R_o/R_i from 13 to 32 (Table 3). This change is consistent with the notion that R_i decreases during exposure to an increase in $[K^+]$.

The depolarization of the V_{sc} and its secondary effects at one or more membrane barriers after increasing the stromal K^+ concentration accounts for part of the anomalous change of R_o/R_i since by voltage clamping V_o the expected increase of R_o/R_i was initially observed (Fig. 3). At present it is not possible to distinguish between the inherent voltage sensitivity of the apical membrane Cl^- permeability and secondary effects on membrane conductance (constant-field predicted or dependent on cellular Cl^- accumulation). These changes do not necessarily need to be large in absolute terms in order to evoke the anomalous response of R_o/R_i if, as suggested above, the partial K^+ conductance of the basolateral membrane is smaller than one.

It is interesting to note that R_o/R_i decreased secondarily even at constant V_o. However, the final stable values for R_o/R_i were greater during the voltage clamping of V_o. This time-dependent behavior of R_o/R_i suggests that there are secondary changes occurring at one or more than one membrane barrier. A morphologically based electrical equivalent of this epithelium includes distributed effects arising from the lateral intercellular spaces which contribute to the basolateral membrane resistance. It is conceivable that increasing stromal-side K^+ to 25 mM causes the basolateral membrane resistance to decrease, intracellular potential difference to depolarize and intracellular Cl^- activity to increase. Cell swelling would follow which results in a large increase in lateral space resistance which in part offsets the expected decrease of basolateral resistance. Therefore, R_o/R_i eventually decreases; however, the final level is higher during clamping since the apical membrane Cl^- conductance may be more constant. Furthermore, the postulated distributed effects of increasing the lateral intercellular space resistance are consistent with the small increases of transepithelial electrical conductance (*cf.* Tables 2*a*, 4 and 5).

The I_{sc} did not decrease at an elevated stromal K^+ concentration as much as after exposure to Ba^{2+} although the V_{sc} (i.e. one part of the driving force for apical membrane Cl^- exit) decreased by about the same amount. These two observations suggest that the Na/Cl symport in the basolateral membrane of the frog cornea requires, in addition, K^+. The stoichiometry of an analogous symport (Na/K/2Cl) has been described in the thick ascending limb of Henle's loop in the kidney (Schlatter et al., 1983). Accordingly, intracellular Cl^- concentration may increase more with 25 mM stromal K^+ than during incubation with Ba^{2+}. Thus the decreases of R_o/R_i may be similar after 25 mM K^+ and Ba^{2+} (*compare* Figs. 1 and 2, lower). The putative larger increase in intracellular Cl^- with 25 mM K^+ would partially offset the decrease in the electrochemical driving force for Cl^- exit resulting in a smaller decrease of the I_{sc} with 25 mM K^+ than with 2 mM Ba^{2+}.

The effects on R_o/R_i of voltage clamping V_t at various desired levels indicates that this parameter changes in accordance with our current understanding of the mechanism of net transepithelial Cl^- transport. Depolarizing V_o decreases R_o/R_i and the converse is observed after hyperpolarization of V_o. These effects are consistent with constant-field predicted changes of the mean Cl^- activity in the cytoplasm bathing the apical membrane. In view of the problems in associating changes of R_o/R_i with any changes of the individual membrane resistances, the changes of R_o/R_i appear to result in part from alterations in the apical Cl^- and any possible basolateral Cl^- membrane conductances since the stepwise removal of Cl^- from the bathing solution muted these changes.

There are a number of other complications which may interfere with the interpretation of what appears to be in part voltage-dependent behavior of

apical Cl^- conductance. We cannot disregard the possibility of nonconservative Cl^- efflux and influx across the apical and basolateral membranes for some time after perturbing V_t and/or polarization effects (i.e. changes of electromotive forces across cell membranes). During these periods, K^+ and/or Cl^- flow across the basolateral membrane may change the conductance of this membrane as well. Furthermore, any voltage sensitivity of the basolateral membrane ionic conductance cannot be excluded.

The results shown in Tables 3 and 4 indicate that the changes in V_{sc} upon elevating stromal K^+ were significantly larger in Cl-free Ringer's than in NaCl Ringer's. This difference suggests that a fraction of the basolateral membrane conductance may be accounted for by a Cl^--conductive pathway: the large apical membrane Cl^{-1} conductance, however, prevents any quantification of this possible pathway. It is important to point out that even in the absence of Cl^- on both sides of the cornea (i.e. $I_{sc} \simeq 0$), the V_{sc} has a value somewhat smaller than the equilibrium potential value for K^+ (i.e. 94 mV) across the basolateral membrane (Reuss et al., 1983). Under this condition, the slope of the relationship between V_{sc} and stromal K^+ concentration is less than the predicted Nernstian slope between 2.5 and 25 mM K^+. Thus, a significant basolateral membrane conductance for ions other than K^+ and Cl^- is conceivable, or it is possible that the K^+ conductance is voltage sensitive. Another conductance appears to be due to Na^+ since substitution of Na^+ with *n*-methyl-D-glucamine in Cl-free Ringer's hyperpolarized V_{sc} by 6 mV. This result is consistent with the earlier suggestion of Na^+ recycling between the cellular compartment and the stromal bathing solution based on measurements of the effects of ouabain on respiratory rates in Cl-free Ringer's (Candia & Reinach, 1982). Finally, our results indicate a small contribution of an electrogenic Na/K pump in the frog cornea to the basolateral membrane potential similar to that of other epithelia in which it was found that incubation with ouabain depolarized the basolateral membrane by less than 10 mV before changes in ionic distribution appear likely (Reuss et al., 1979; Nagel, 1980).

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References

Bello-Reuss, E. 1982. Electrical properties of the basolateral membrane of the straight portion of the rabbit proximal renal tubule. *J. Physiol. (London)* **326:**49–63

Biagi, B., Kubota, T., Sohtell, M., Giebisch, G. 1981. Intracellular potentials in rabbit proximal tubules perfused in vitro. *Am. J. Physiol.* **240:**F200–F210

Candia, O.A., Reinach, P.S. 1982. Thermodynamic analysis of active sodium and potassium transport in the frog corneal epithelium. *Am. J. Physiol.* **242:**F690–F698

Candia, O.A., Reinach, P.S., Alvarez, L. 1984. Amphotericin B-induced active transport of K^+ and the Na^+-K^+ flux ratio in frog corneal epithelium. *Am. J. Physiol.* **247:**C454–C461

Greger, R., Schlatter, E. 1983. Properties of the lumen membrane of the cortical thick ascending limb of Henle's loop of rabbit kidney. *Pfluegers Arch.* **396:**315–324

Kirk, K.L., Dawson, D.C. 1983. Basolateral potassium channel in turtle colon. *J. Gen. Physiol.* **82:**297–313

Kirk, K.L., Halm, D.R., Dawson, D.C. 1980. Active sodium transport by turtle colon via an electrogenic Na-K exchange pump. *Nature (London)* **287:**237–239

Koeppen, B.M., Biagi, B.A., Giebisch, G.H. 1983. Intracellular microelectrode characterization of the rabbit cortical collecting duct. *Am. J. Physiol.* **244:**F35–F47

Lewis, S.A., Wills, N.K., Eaton, D.C. 1978. Basolateral membrane potential of a tight epithelium: Ionic diffusion and electrogenic pumps. *J. Membrane Biol.* **41:**117–148

McLennan, W.L., Machen, T.E., Zeuthen, T. 1980. Ba^{2+} inhibition of electrogenic Cl^- secretion in in vitro frog and piglet gastric mucosa. *Am. J. Physiol.* **239:**G151–G160

Nagel, W. 1978. Effects of antidiuretic hormone upon electrical potential and resistance of apical and basolateral membranes of frog skin. *J. Membrane Biol.* **42:**99–122

Nagel, W. 1979. Inhibition of potassium conductance by barium in frog skin epithelium. *Biochim. Biophys. Acta* **552:**346–357

Nagel, W. 1980. Rheogenic sodium transport in a tight epithelium, the amphibian skin. *J. Physiol. (London)* **302:**281–295

Nagel, W., Reinach, P. 1980. Mechanism of stimulation by epinephrine of active transepithelial Cl^- transport in isolated frog cornea. *J. Membrane Biol.* **56:**73–79

Nielsen, R. 1979. A 3 to 2 coupling of the Na-K pump responsible for the transepithelial Na transport in frog skin disclosed by the effect of Ba. *Acta Physiol. Scand.* **107:**189–191

O'Neil, R., Sansom, S. 1984. Characterization of apical cell membrane Na^+ and K^+ conductances of cortical collecting duct using microelectrode techniques. *Am. J. Physiol.* **247:**F14–F24

Planelles, G., Teulon, J., Anagnostopoulos, T. 1981. The effects of barium on the electrical properties of the basolateral membrane in proximal tubule. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **318:**135–141

Reuss, L. 1979. Electrical properties of the cellular transepithelial pathway in *Necturus* gallbladder: III. Ionic permeability of the basolateral cell membrane. *J. Membrane Biol.* **47:**239–259

Reuss, L., Bello-Reuss, E., Grady, T. 1979. Effects of ouabain on fluid transport and electrical properties of *Necturus* gallbladder. *J. Gen. Physiol.* **73:**385–402

Reuss, L., Cheung, L.Y., Grady, T.P. 1981. Mechanisms of cation permeation across apical cell membrane of *Necturus* gallbladder: Effects of luminal pH and divalent cations on K^+ and Na^+ permeability. *J. Membrane Biol.* **59:**211–224

Reuss, L., Reinach, P., Weinman, S.A., Grady, T.P. 1983. Intracellular ion activities and Cl^- transport mechanisms in bullfrog corneal epithelium. *Am. J. Physiol.* **244:**C336–C347

Schlatter, E., Greger, R., Weidtke, C. 1983. Effect of high ceiling diuretics on active salt transport in the cortical thick ascending limb of Henle loop of rabbit kidney: Correlation of chemical structure and inhibitory potency. *Pfluegers Arch.* **396:**210–217

Van Driessche, W., Zeiske, W. 1980. Ba^{2+}-induced conductance fluctuations of spontaneously fluctuating K^+ channels in the apical membrane of frog skin (*Rana temporaria*). *J. Membrane Biol.* **56:**31–42

Welsh, M.J. 1983. Barium inhibition of basolateral membrane potassium conductance in tracheal epithelium. *Am. J. Physiol.* **244:**F639–F645

Wills, N.K., Zeiske, W., Van Driessche, W. 1982. Noise analysis reveals K^+ channel conductance fluctuations in the apical membrane of rabbit colon. *J. Membrane Biol.* **69:**187–197

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