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Basolateral Membrane K Permselectivity and Regulation in Bullfrog Cornea Epithelium

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Summary. In the isolated bullfrog cornea epithelium, under short-circuit conditions the regulation of the K permeability of the basolateral membrane was studied with conventional and K-selective microelectrodes in Cl-free Ringers. In Cl-free Ringers, the transcellular current is less than 1 $\mu A/cm^2$, allowing estimation of the basolateral membrane electromotive force from measurements of the membrane voltage (V_{sc}). The apparent basolateral membrane K conductance was determined from measurements of the effects of single ion substitutions of K for Na on the V_{sc}. An increase of K from 2.5 to 25 mM on the stromal side depolarized the membrane voltage by 29 mV, whereas additional increases to 56 and 100 mM resulted in depolarizations consistent with a Nernstian prediction. In the range between 25 and 56 mM K, these decreases in membrane voltage were smaller after either decreasing the stromal-side pH from 8.1 to 7.2 or substitution of sulfate with gluconate. In contrast, preincubation with 0.1 mM ouabain did not change the membrane voltage depolarizations over any of the K ranges between 2.5 and 100 mM. Equivalent circuit analysis, based on the effects of nystatin on the electrical parameters, was used to validate the changes in the apparent basolateral membrane K conductance following increases in [K], substitution of SO_4 with gluconate and Na:K pump inhibition. An increase in the [K] to 120 mM decreased the basolateral membrane resistance nearly three-fold, whereas gluconate substitution resulted in a 2.5-fold increase of the basolateral membrane resistance. This resistance increased an additional 2-fold after exposure to 5 mM Ba. However, exposure to 0.1 mM ouabain had no significant effect on this resistance. Therefore, there is an agreement between the results of circuit analysis and the magnitude of membrane voltage depolarization resulting from increases in [K], gluconate substitution and pump inhibition with ouabain. Na:K pump inhibition with ouabain caused the K activity to decline slightly after 30 min from 98 ± 7 to 83 ± 8 mM, which is consistent with a small basolateral membrane Na conductance. The estimated K permeability ranged from 3.7×10^{-7} to 1.1×10^{-6} cm/sec. The less than Nernstian predicted decline of the membrane voltage between 2.5 and 25 mM K and the small basolateral membrane Na conductance suggest that the basolateral membrane is also permeable to another unknown ion.

Key Words active Cl transport · bullfrog · K permeability · barium · membrane voltage · intracellular K activity · short-circuit current · ouabain

Introduction

In the isolated bullfrog cornea epithelium, net Cl transport from the stroma to the tear-side bathing solution accounts for more than 90% of the short-circuit current (Zadunaisky, 1966). The steps in this process include coupled Na:Cl uptake from the stromal bathing solution, against an electrochemical gradient across the basolateral membrane, followed by Cl electrodiffusion into the tears across the apical membrane. This membrane has a large Cl and very small K permselectivity (Reuss et al., 1983). The electrical driving force eliciting Cl electrodiffusion is in part a consequence of net K efflux across the basolateral membrane into the stromal bathing solution since the K channel blocker, Ba, depolarized the membrane voltage and inhibited net Cl transport by 62% (Reinach & Nagel, 1985). The purpose of the present study was to identify some of the regulatory parameters of the basolateral membrane K conductance.

Our results show that the apparent basolateral membrane K conductance is inhibited by a decrease in pH, a sulfate with gluconate substitution but is unchanged subsequent to inhibition of the Na:K pump with ouabain. The effect of acidification on the membrane voltage is also consistent with an increase of the Na conductance. Nystatin was used to perform circuit analysis and to evaluate either the effects of increases in the [K], gluconate substitution on the stromal-side or exposure to ouabain on the membrane parameters. We found that there is a correspondence between the changes in membrane resistance and membrane voltage depolarizations produced by K for Na substitutions after either increases in the [K], gluconate substitution on the stromal side or exposure to 0.1 mM ouabain. Measurements of the effects of ouabain on intracellular K activity are consistent with the notion of a finite

Table 1. Effect of 0.1 mM ouabain on the V_{sc} change resulting from an increase in [K] ($n = 11$)

	[K] mM		ΔV_{sc}
	2.5	25	
Control	−79 ± 1	−51 ± 4	28 ± 4
Ouabain	−62 ± 2	−36 ± 1	26 ± 2

basolateral membrane Na conductance since the K activity only declined slightly 30 min after exposure to ouabain.

Materials and Methods

Corneas were removed from double-pithed bullfrogs (*Rana catesbeiana*) and mounted horizontally in a modified Ussing chamber for simultaneous measurements of their transepithelial and intracellular electrical parameters (Reinach & Nagel, 1985). Single-barreled K-selective microelectrodes were fabricated according to the method previously described for K-selective microelectrodes (Garcia-Diaz et al., 1985). The osmolality of the Ringers' solution was 220 mOsm, the pH was 8.1 and were equilibrated with room air. Compositions were (in mM): (i) Na_2SO_4-Na 110, SO_4 61, K 2.5, Mg 1, Ca 5, HEPES 3.5, glucose 5 and osmolality adjusted to 220 mOsm with sucrose addition; (ii) Na-gluconate-equimolar substitutions of Na, Ca (5 mM) salts with corresponding gluconate salts whereas Mg acetate was used in place of Mg_2SO_4 (iii) Elevated K^+ either K_2SO_4 or K-gluconate was substituted for Na_2SO_4 on an equimolar basis to obtain K concentrations of 2.5, 25, 56 and 100 mM (iv) "Cell-like" Ringer K 120, Na 20, Cl 56, Mg 1, Ca 5, SO_4 75, HEPES 3.5 and glucose 5. (v) NaCl Na 110, K 2.5, Ca 1, Mg 1, Cl 114, glucose 5 and HEPES 3.5. The following drugs were used: ouabain octahydrate, isobutylmethylxanthine (IBMX) and nystatin (Sigma Chemical Co., St. Louis, MO). A nystatin stock solution was made by dissolving 5 mg of the ionophore in 1 ml of ethanol and dimethylsulfoxide (vol/vol, 9:1). A 1:50 dilution was made of this stock to obtain a working concentration of 590 USP units/ml. All results are reported as means ± SEM. Statistical significance was evaluated using paired Student's t test.

List of Symbols

1. I_{sc} = transcellular current under short-circuit conditions
2. g_t = transepithelial conductance
3. V_{sc} = intracellular membrane voltage under short-circuit conditions (tear : ref)
4. fR_o = relative resistance of apical membrane:

$$R_a/(R_a + R_b)$$

5. V_K = intracellular membrane voltage measured with a K-selective microelectrode
6. $\alpha = g_b$ (basolateral membrane conductance)$/g_a$ (apical membrane conductance)

Results

In Na_2SO_4 Ringers, the I_{sc} is almost equal to zero (i.e., between 1 and 0 $\mu A/cm^2$) since the net Na flux is very small (Candia & Askew, 1968). The I_{sc} is rate limited by the immeasurably small apical membrane Na conductance (Reuss et al., 1983). In this Ringer's solution, the electrical resistance of the apical membrane is at least one order of magnitude larger than the resistance of the basolateral membrane (Reinach & Nagel, 1985; Candia & Cook, 1986). Therefore, in Cl-free Ringers and under short-circuit conditions measurements of the V_{sc} approximate those of the electromotive force across the basolateral membrane since the I_{sc} is very small and fR_o is between 90 and 100%.

In other epithelia, there appears to be a relationship between Na : K pump activity and the apparent basolateral membrane K conductance. A decline of the apparent basolateral membrane K conductance after Na : K pump inhibition with ouabain was observed in tissues mediating significant rates of net ion transport (Helman, Nagel & Fisher, 1979; Reuss, Bello-Reuss & Grady, 1979; Matsumura et al., 1984; Wang et al., 1984; Messner et al., 1985), whereas in the absence of net ion transport, inhibition of the Na : K pump had no effect on the basolateral membrane K permeability (Kubota, Biagi & Giebisch, 1983).

We determined in the same group of 11 corneas the effect of Na : K pump inhibition with ouabain on the apparent basolateral membrane K conductance from a comparison of the depolarizations of the V_{sc} after an increase of the stromal-side [K] between 2.5 and 25 mM. Under control conditions, the V_{sc} depolarized by 28 ± 4 mV after 10 min (*cf.* Table 1). This change was completely reversible with the same time course. Subsequent exposure to 0.1 mM ouabain depolarized the V_{sc} by 17 ± 5 mV but had no significant effect on fR_o. An interpretation of this lack of change in fR_o is that measurements of fR_o may not resolve changes in cell membrane parameters. Elevation of the K from 2.5 to 25 mM decreased the V_{sc} by 26 ± 2 mV, which is not significantly different than the control change (*cf.* Table 1). A typical experiment indicating the time courses for these changes as well as an increase to 100 mM K is shown in Fig. 1. It should be noticed that the onset of the depolarization of the V_{sc} after any substitution or drug addition to the stromal-side is less than 1 min; however, owing to diffusion delays through unstirred layers attainment of a stable value requires a 10-min delay. Throughout the control periods and during exposure to ouabain as well as changes in [K], the I_{sc} was stable at less than 1 $\mu A/cm^2$. Therefore, ouabain did not appear to alter the apparent basolateral membrane K conductance,

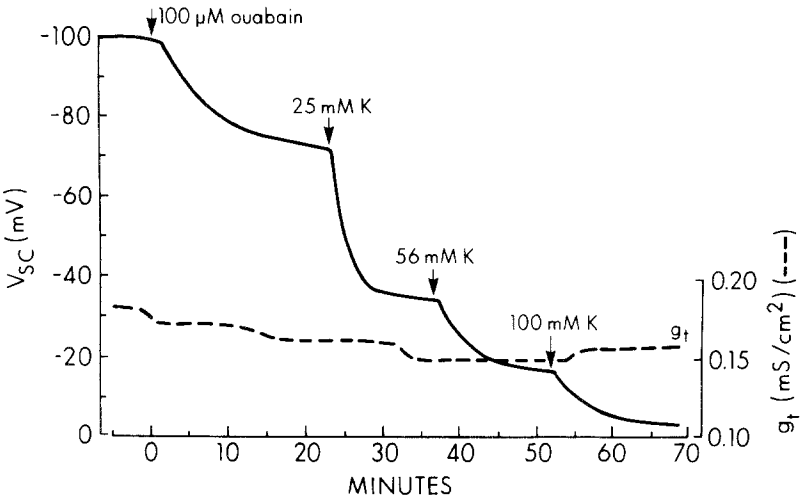

Fig. 1. Typical consecutive effects of ouabain and increases in stromal-side [K] (Na_2SO_4 Ringer's solution) on the V_{sc} and g_t. The I_{sc} was nearly equal to zero and unchanged by exposures to ouabain and increases in the [K]. Step isosmotic substitutions of Na_2SO_4 with K_2SO_4 Ringers on the stromal-side is indicated by arrows

which is in agreement with the lack of an effect by ouabain in the proximal tubule in the absence of net ion transport (Kubota et al., 1983).

In other epithelia, there is evidence showing that acidification of the bathing solution results in a decrease of the K conductance (Reuss, Cheung & Grady, 1981; Kubota et al., 1983). The pH dependency of the apparent basolateral membrane K conductance was considered by comparing in the same cornea the depolarizing effects on the V_{sc} of increasing the [K] from 25 to 56 mM first at pH = 8.1 and then 7.2 (*cf.* Fig. 2*A*). In eight corneas (pH 8.1), the V_{sc} in Na_2SO_4 Ringers was 80 ± 4 mV with 2.5 mM K and decreased to 40 ± 3 mV following an increase of the [K] to 25 mM. Subsequent increase of the [K] to 56 mM caused the V_{sc} to depolarize to 17 ± 3 mV. Re-exposure to 25 mM K resulted in hyperpolarization to a stable value of 44 ± 3 mV, where upon replacement with Na_2SO_4 Ringers containing 25 mM K (pH 7.2) resulted in a 9-mV depolarization to 35 ± 3 mV ($P < 0.05$). Subsequent increase to 56 mM K resulted in depolarization to 18 ± 3 mV. Therefore, the depolarization elicited by increasing in the same group of corneas the [K] from 25 to 56 mM decreased from 23 ± 2 mV to 17 ± 2 mV. This decline is the result of the 9-mV depolarization subsequent to changing the Ringer's pH from 8.1 to 7.2. This change is consistent with a decrease of the apparent basolateral membrane K conductance, an increase of the Na conductance and/or a H^+ conductance.

Recently it has become apparent that the isosmotic substitution of Cl with gluconate on the serosal side in the isolated toad urinary bladder, frog skin and rabbit urinary bladder inhibits the basolateral membrane K conductance (Lewis, et al., 1985; Klemperer, Garcia-Diaz & Essig, 1986; Chen & Lewis, 1987). The gluconate dependency of the apparent basolateral membrane K conductance was determined in the same cornea by comparing the depolarizing effects on the V_{sc} of increasing the [K] between 25 and 56 mM (cf. Fig. 2*b*). In Na_2SO_4 Ringers, the V_{sc} decreased from 40 ± 3 mV to 17 ± 2 mV (n = 5) after 15 min. This depolarization effect was completely reversible in the same time span after restoring 25 mM K. Substitution of Na_2SO_4 with Na-gluconate Ringers containing 25 mM K depolarized the V_{sc} to 34 ± 2 mV, which was followed by an additional depolarization to 23 ± 1 mV after increasing the [K] to 56 mM. Therefore, gluconate appears to inhibit the apparent basolateral membrane K conductance since the depolarization of the V_{sc} resulting from increasing the [K] from 25 to 56 mM in gluconate-containing Ringers was 52% smaller than in sulfate-containing Ringers. In the same group of four corneas, the possibility was con-

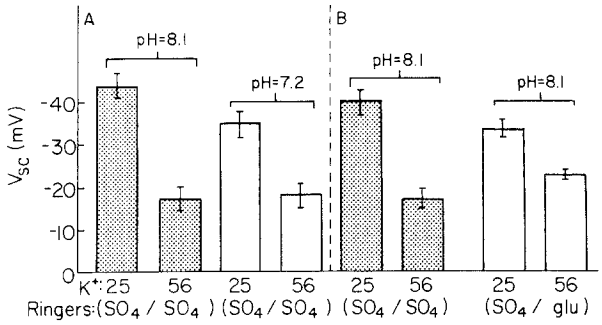

Fig. 2. (*A*) Comparison of the consecutive effects in the same cornea of increases in stromal-side [K] between 25 and 56 mM on the V_{sc} at pH = 8.1 (dotted bars) and pH = 7.2 (open bars) (n = 8). (*B*) Comparison of the consecutive effects in the same cornea of increases in stromal-side [K] between 25 and 56 mM on the V_{sc} in Na_2SO_4 Ringers (dotted bars) and Na-gluconate Ringers (open bars) (n = 5)

sidered of cAMP or Ca mediated regulation of the basolateral membrane K conductance. This was determined from a comparison of the ΔV_{sc} elicited by increasing the stromal-side [K] from 25 to 56 mM before and after exposure to first 1 mM quinidine and then 0.1 mM epinephrine. In those corneas where ΔV_{sc} was reversible within 10 min. after restoring the [K] to 25 mM, they were then exposed to 1 mM quinidine on the stromal-side (Table 2). Quinidine had no effect on the V_{sc} and the depolarization of the V_{sc} was similar to the control change. Restoration of 25 mM K caused the V_{sc} to repolarize to about the same level previous to quinidine exposure. Subsequent exposure to epinephrine on the stromal side also had no effect on both the V_{sc} and the change of the V_{sc} after increasing the [K] to 56 mM. Therefore, neither cAMP nor Ca mediated regulation of the basolateral membrane conductance is apparent in the absence of net ion transport.

Another approach to evaluating the apparent conductance of the basolateral membrane is to perform equivalent circuit analysis. This approach is advantageous since it is possible to evaluate the shunt and apical membrane resistances as well. In Na_2SO_4 Ringers, this technique has also the potential of being more sensitive to any changes in the basolateral membrane conductance parameters than measurements of the fR_o which exceed 90%. Previous circuit analyses in the cornea relied on the slow and sometimes not completely reversible effects of either adenosine or amphotericin B on the electrical parameters (Reuss et al., 1983; Candia & Cook, 1986). In particular, their variable reversibility prevents each cornea from serving as its own control in any experimental maneuver since it is necessary that the agent's effects have a rapid onset followed by complete recovery to a control level during washout. We considered instead the effects of nystatin under short-circuit conditions to perform equivalent circuit analyses since in other epithelia its effects are rapidly and fully reversible (Lewis et al., 1977; Wills, Lewis & Eaton, 1979).

Following the precautions used by Lewis et al., to selectively increase the apical membrane conductance subsequent to nystatin exposure, the tear and stromal side solutions contained "cell-like" Ringers and Na_2SO_4 Ringers, respectively. "Cell-like" Ringers mimicked more closely the intracellular milieu under control conditions so that an indirect effect of nystatin on the basolateral membrane due to changes in intracellular composition could be minimized (Lewis et al., 1977). Nystatin, at a con-

Table 2. Effect of 1 mM quinidine and 0.1 mM epinephrine on the changes of the V_{sc} caused by increasing the [K] from 25 to 56 mM (n = 4)

[K]	Control	Quinidine	Epinephrine
25	−34 ± 3	−29 ± 3	−34 ± 4
56	−20 ± 1	−11 ± 6	−21 ± 2
ΔV_{sc}	13 ± 3	18 ± 4[a]	14 ± 5[a]

[a] NS.

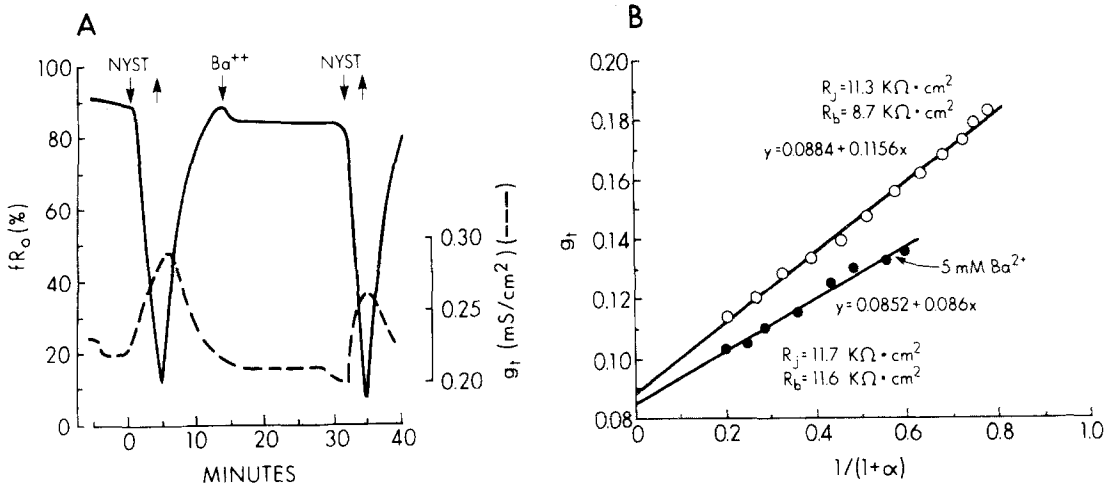

Fig. 3. (*A*) Time course of the consecutive effects of tear-side 590 U/ml nystatin (at downward arrows) and 5 mM Ba on fR_o and g_t: Corneas were short circuited and bathed in "cell-like" Ringer's solution only on the tear side, whereas the stromal-side contained Na-gluconate Ringer's solution. The rapid and complete reversible effects of nystatin permitted comparisons to be made in the same cornea of derived values for the circuit parameters in the control and experimental conditions. (*B*) The relationship between g_t and $1/(1 + \alpha)$ in the experiment shown in *A*. The slope of the line represents the basolateral membrane conductance, g_b and the y intercept is the junctional conductance, g_j

centration of 590 units/ml, was used to obtain rapid and large increases of the I_{sc}.

In Fig. 3*A*, are shown the time-dependent effects of nystatin on g_t and fR_o before and after exposure to 5 mM Ba in Na-gluconate Ringers on the stromal side. After a 4-min exposure to nystatin, g_t increased from 0.21 to 0.29 mS/cm^2 and fR_o decreased from 90 to 10%. In other experiments, neither fR_o nor g_t changed any further if the nystatin exposure time was extended for 10 min. The duration of the washout phase of nystatin from the tear-side bathing solution was 11 min and the changes in the electrical parameters were completely reversible. After an 18-min pre-exposure to Ba, which slightly decreased the fR_o and g_t, nystatin was added to "cell-like" Ringer on the tear side. Within 4 min g_t increased from 0.20 to 0.27 mS/cm^2 and fR_o decreased from 85 to 8%. The sets of values from Fig. 3*A* of g_t and fR_o during the exposure periods to nystatin were replotted in Fig. 3*B* with g_t as a function of $1/(1 + \alpha)$; where α is equal to the ratio: g_b/g_a. This ratio is obtained directly from fR_o since it is equal to $fR_o/(1 - fR_o)$. The plots shown in Fig. 3*B* were performed to determine if the changes in the electrical parameters were in accord with the function: $g_t = g_j + g_b\ 1/(1 + \alpha)$ (Lewis et al., 1977); where g_j and g_b represent the junctional (ordinate intercept) and basolateral membrane (slope) conductances, respectively. The plots in Fig. 3*B* are linear with correlation coefficients of 1.00 and 0.99 during the control of Ba periods, respectively. This agreement with the above indicated formulation also indicates that only the apical membrane conductance is increased by nystatin. The results of the effects of 5 mM Ba in Na-gluconate on the stromal side of six corneas on R_a, R_b and R_j are shown in Table 3. Ba increased the basolateral membrane resistance, R_b, five-fold relative to the SO_4 control for the gluconate substitution experiment, whereas neither the apical membrane resistance, R_a, nor the junctional resistance, R_j were changed. Additional validation of the technique is that the large increase of R_b after Ba is in agreement with the previously described qualitative effects of Ba on this parameter (Reinach & Nagel, 1985; Candia & Cook, 1986). We also considered the effects of a SO_4 substitution on the stromal side with gluconate alone since this substitution decreased the apparent basolateral membrane K conductance. The results shown in Table 3 indicate that R_a, R_b increased two-fold and 2.5-fold, respectively, but as with Ba, R_j remained unaltered.

The effects were also considered of 0.1 mM ouabain and increases of stromal-side [K] on the membrane parameters (*cf.* Table 3). Exposure to ouabain had no significant effect on any of the circuit parameters whereas increasing the [K] from 2.5 to 120 mM nearly decreased R_b three-fold without having a significant effect on either R_a or R_j. It is noteworthy that all of the changes in the apparent basolateral K conductance, determined from the magnitudes of the voltage depolarization resulting from increasing the [K] after substituting SO_4 with gluconate and K for Na, are in agreement with the results of circuit

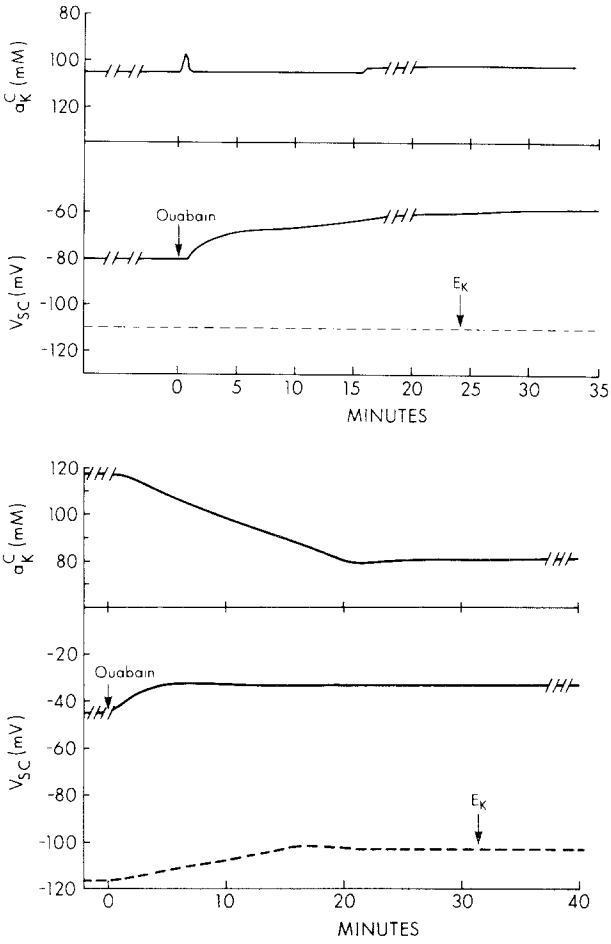

Fig. 4. (*top*) No change of intracellular K activity after ouabain exposure. Simultaneous recordings of the differential output between V_K and V_{sc} (i.e., intracellular K activity, a_K and V_{sc} (bottom) with respect to time. Corneas were bathed in Na_2SO_4 Ringer's solution with 5×10^{-4} M IBMX on the stromal side to increase the apical membrane Cl conductance. During the interruptions in the tracings, Na_2SO_4 Ringer's solution was transiently substituted with NaCl Ringer's solution on the tear side to validate an impalement (*see* Results). E_K is the calculated equilibrium potential. (*bottom*) Decrease of intracellular K activity after ouabain. The protocol and conditions were identical to those described in Figure 4 (top) legend, except that there was a gradual decline of a_K which persisted beyond the time of depolarization of the V_{sc} before restabilization. Note that in both experiments a_K stabilized at a value greater than what is predicted by the Nernst equation

Table 3. Effects of stromal-side changes on equivalent circuit parameters

Tear/stroma	n		R_a	R_b (kΩ · cm²)	R_j
1) Δ cr/SO_4	[a]		98 ± 50	5.2 ± 0.4	6.6 ± 1.6
Δ cr/glu	6	ΔR =	179 ± 56	13.1 ± 3.0	7.3 ± 1.5
			86 ± 40	7.3 ± 1.7	0.3 ± 0.9
			$P < 0.05$	<0.001	<0.5
2) Δ cr/glu	[a]		245 ± 141	14.6 ± 3.3	7.3 ± 0.5
Δ cr/glu + 5 mM Ba	6	ΔR =	168 ± 73	25.0 ± 4.7	7.7 ± 0.8
			16 ± 36	9.5 ± 2.9	0.4 ± 0.4
			$P > 0.5$	<0.001	>0.4
3) Δ cr/SO_4			96 ± 46	7.7 ± 3.0	7.6 ± 1.6
Δ cr/SO_4 + 0.1 mM ouabain	7	ΔR =	117 ± 58	5.2 ± 2.0	8.0 ± 1.8
			11 ± 15	2.5 ± 1.2	0.5 ± 0.5
			$P > 0.5$	<0.1	>0.4
4) Δ cr/SO_4 (2.5 mM K)			108 ± 38	16.2 ± 2.7	4.8 ± 0.6
Δ cr/SO_4 (120 mM K)	7	ΔR =	144 ± 37	6.3 ± 1.3	4.1 ± 0.6
			35 ± 15	10.4 ± 3.0	0.7 ± 0.7
			$P > 0.5$	<0.05	>0.4

[a] Variability results from different corneas in each of these studies.
Δ, "cell-like" Ringer.

analyses. Similarly the lack of an effect with ouabain is in accord with the lack of a change in membrane voltage depolarization resulting from increasing the [K] after exposure to ouabain.

The intracellular K activity was measured of corneas bathed in Na_2SO_4 Ringers. As has been previously described, intracellular K activity was calculated from simultaneous recordings of V_{sc} and V_K in neighboring cells with single-barrel microelectrodes (Garcia-Diaz et al., 1985). The difference, $V_{sc} - V_K$, is used to calculate intracellular K activity. To assess the validity of an impalement, substitutions of Na_2SO_4 with NaCl Ringers were performed for less than 3 min on the tear-side in the presence of 5×10^{-4} M IBMX on the stromal side. IBMX was added to increase the apical membrane Cl conductance resulting in larger changes of the V_{sc} and fR_o during a transient tear-side substitution with NaCl Ringers. Only those impalements were considered acceptable which showed: (i) hyperpolarization of the V_{sc} and decreases of fR_o that agreed within ±5% of one another as measured with the conventional and K-selective microelectrodes; (ii) since we assumed that intracellular K activity was unaffected by this type of substitution, any sustained change of the differential trace had to be less than 4 mV and any transient change larger than this value had to last less than 20 sec. In experiments satisfying these criteria, ouabain was then perfused through the stromal side during the continuous measurement of all parameters. Two representative traces from nine separate corneas, showing quite different results, are shown in the top and bottom panels of Fig. 4. Only the effects of ouabain on V_{sc} and the intracellular K activity are shown, since fR_o, I_{sc} and g_t were all unchanged. The two traces shown in the figure are from two corneas, indicating the extremes of the effects of ouabain on intracellular K activity; pump inhibition had no significant effect since the K activity declined by only 2 mM from 105 to 103 mM subsequent to a 17-mV depolarization of the V_{sc}; (bottom) K activity declined from 118 mM, at the same time that the V_{sc} depolarized by 12 mV, reaching a final value of 80 mM subsequent to stabilization of the decline in the V_{sc}. In the nine corneas, the stable mean values of intracellular activity prior and 30 min subsequent to ouabain exposure were 98 ± 7 and 83 ± 8 mM, respectively ($P < 0.05$). In all cases it should be noted that the stable value of intracellular K activity subsequent to ouabain exposure was always significantly larger than what is predicted for an equilibrium distribution.

Discussion

We characterized the conductance of the basolateral membrane from measurements of the V_{sc} in Na_2SO_4 Ringers since the I_{sc} is between 1 and 0 μA/cm² which suggests that current flow across the ba-

solateral membrane results in less than a 10-mV depolarization of the electromotive force across this membrane. This procedure for evaluating the conductance properties of the basolateral membrane is an alternative to performing circuit analyses. Circuit analyses is based on the analysis of the effects of either epinephrine, adenosine or amphotericin B, on the electrical parameters (Nagel & Reinach, 1980; Reuss et al., 1983; Candia & Cook, 1986). The validity of circuit analysis depends on assuming that only a single element in the equivalent circuit is modified by any one of these agents. It was previously shown that the selectivity of these agents in modifying only the apical membrane conductance is time dependent in that with longer periods of exposure secondary effects are also possible. A second limitation of circuit analysis is that the reversibility of the above-mentioned agents is slow and not always complete, which makes it questionable whether or not it is meaningful to use each cornea as its own control. Therefore, it was desirable to examine more directly the properties of the basolateral membrane from measurements of the V_{sc} in Na_2SO_4 Ringers.

Our results show that the basolateral membrane has a significant K conductance but that in addition there may be other parallel pathways. Indication that the basolateral membrane is not K permselective stems from the observation that increases in the stromal-side [K] between 2.5 and 25 mM resulted in less than a Nernstian predicted change of the V_{sc}. Another possible conductive pathway includes Na^+ based on the slow rate of decline in intracellular K activity subsequent to ouabain exposure and the previously measured small but significant hyperpolarization of the V_{sc} subsequent to the removal of Na in Cl-free Ringers (Reinach & Nagel, 1985). The diminution of the depolarizing effect of an increase in [K] between 25 and 56 mM on the V_{sc} after acidification of the stromal-side bathing solution has several interpretations. This effect is consistent with a pH-dependent K^+ and/or Na^+ H^+ conductances.

The suggestion of a substantial basolateral membrane H^+ conductance is not novel in view of the finding that in frog skin the basolateral membrane has an appreciable H^+ conductance (Helman, 1987). Another indication of other conductive pathways besides K^+ is that the calculated value for E_K, based on our mean measurement of intracellular K activity, is in many cases larger than the V_{sc}. The fact that the change in the V_{sc} was Nernstian between 25 and 100 mM K is consistent with a voltage-dependent K conductance and/or that it is not possible to resolve other smaller conductances since K is the more prominent cation.

The regulatory parameters of the basolateral membrane K conductance which we considered were Na : K pump activity, changes in putative intracellular cAMP levels, changes in putative intracellular Ca level, cell volume, stromal-side pH and SO_4 with gluconate substitution. Inhibition of the Na : K pump with 0.1 mM ouabain, did not change the depolarizing effect on the V_{sc} of an increase in the [K] from 2.5 to 25 mM. Similarly, neither preexposure to 1 mM quinidine nor 0.1 mM epinephrine affected the depolarizing effect on the V_{sc} of an increase in K from 25 to 56 mM. The lack of an effect with ouabain is consistent with the notion that in the absence of significant net ion transport changes in the basolateral membrane K conductance do not occur in response to inhibition of the Na : K pump (Schultz, 1981). The lack of an effect by either quinidine or epinephrine are at variance with changes in other epithelia of the basolateral membrane K conductance which are, however, mediating net ion transport. Quinidine and epinephrine-sensitive conductances have been described in the isolated turtle colon and canine tracheal epithelium, respectively (Germann et al., 1986; Smith & Frizzell, 1984). Similarly, cell volume changes resulting from either decreasing the bath osmolality to 140 mOsm or increasing it to 300 mOsm, with sucrose removal and addition, had no effect on any of the baseline parameters measured in Na_2SO_4 Ringer's solution (*unpublished observation*). The only identified parameters that appear to decrease the apparent basolateral membrane K conductance are stromal-side acidification and the substitution of SO_4 with gluconate. An inhibition of the K conductance resulting from acidification has been previously reported in other epithelia (Reuss et al., 1981; Kubota et al., 1983). A number of excitable and nonexcitable K channels in several axon preparations, skeletal muscle and starfish oocytes are reversibly blocked by low external pH. The titratable acid groups regulating this K channel activity appear to be accessible from the cell cytosol since the K currents are only blocked subsequent to acidification of the cell interior (Blatz, 1984). In the cornea, such identification of the site of protonation is not yet possible. Recently it has been demonstrated in the rabbit urinary bladder that the replacement of serosal Cl with gluconate depolarized the basolateral membrane potential without altering intracellular K activity. This effect was attributed solely to a decrease in the basolateral membrane K permeability (Chen & Lewis, 1987). However, in the cornea it is possible that the effects of gluconate on the V_{sc} and its response to changes in [K] are due to a decrease in basolateral membrane K and/or increase of Na permeabilities.

As has been frequently performed in other epi-

thelia, forms of the Goldman equation have been applied to characterize basolateral membrane properties. Provided there are no rheogenic pumps contributing to the membrane voltage, it is possible to use a form of this equation to calculate the basolateral membrane K permeability, P_K (Reuss & Finn, 1975). The transference number, T_K, is calculated:

$$T_K = \Delta V_{sc}/58 \log(c_1/c_2)$$

where ΔV_{sc} is the change of the V_{sc} produced by the substitution of 2.5 with 25 mM K on the stromal side and c_1 and c_2 are the external activities of K before and after the substitution, T_K is equal to 0.45. P_K can be calculated from the following relationship:

$$P_K = \frac{RT\, T_K\, G_b}{z^2\, \bar{c}_K\, F^2}.$$

The range of values of G_b are approximately from 1.9×10^{-4} to 6×10^{-5} S/cm^2 (*cf.* Table 3) after ouabain. The product of T_K and G_b is the partial K conductance: $\bar{c}_k$ is the mean K activity in the membrane (i.e., 18×10^{-6} moles/cm^3) and R, z and F have their usual meanings. The range of values of P_K is between 3.7×10^{-7} and 1.1×10^{-6} cm/sec, similar to a previously reported range calculated with a different formalism and using estimated values of intracellular K activity (Candia & Cook, 1986).

The effects of brief exposure to nystatin under short-circuit conditions were analyzed using equivalent circuit analysis. In the rabbit urinary bladder and colon, these experiments were performed under open rather than short-circuit conditions (Lewis et al., 1977; Wills et al., 1979). It is possible to calculate, in any case, meaningful values for the equivalent under open-circuit conditions since the equivalent only contains linear elements. We chose to perform our measurements under short-circuit conditions since the I_{sc} is another indicator of the time course of the onset and the washout of the nystatin-induced changes in the electrical parameters. Awareness of the time course lessens the likelihood of too long an exposure to nystatin, which could modify more than a single element in the equivalent.

We followed the previously described protocol to minimize the possibility that nystatin modified any other resistance besides the apical membrane (Lewis et al., 1977). A minimal exposure time to nystatin coupled with the use of a "cell-like" Ringer resulted in changes of the electrical parameters that are consistent with a single rather than a multiple change. The function shown in Fig. 3*B* is linear and was unchanged for an additional 10 min beyond the indicated time. It is possible but unlikely that each increase of the apical membrane conductance was accompanied by a corresponding change in the basolateral membrane conductance which maintained a linear relationship between g_t and $1/(1 + \alpha)$. Another indication that the basolateral membrane conductance remained constant is that exposure to nystatin for 10 min from the stromal side had no effect on the electrical parameters. Finally the hydrophobic nature of nystatin makes it unlikely that its addition to the tear side will result in incorporation into the basolateral membrane (Garty, 1984). There is suggestive evidence that nystatin does not alter the paracellular pathway since in another study amphotericin B had no effect on mannitol permeability after 4 min of exposure (Candia, Reinach & Alvarez, 1984). Furthermore, in no case was R_j altered in the current study.

A distinct advantage of the nystatin technique is the rapid and complete reversibility of any changes in the electrical parameters, permitting each cornea to serve as its own control in any experimental maneuver. This makes it easier to identify meaningful changes despite intercorneal variability in the membrane parameters. The changes in the basolateral membrane resulting from either a substitution of SO_4 with gluconate or exposure to ouabain are consistent with the above-mentioned changes in the apparent basolateral membrane conductance. Another indication that the results of circuit analysis are representative of the apparent basolateral membrane K conductance is that the basolateral membrane resistance decreased nearly threefold after increasing the stromal-side K from 2.5 to 120 mM.

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