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Microelectrode studies of amphotericin B on Na^+ and K^+ conductance in bullfrog cornea

Gaspar Carrasquer [1], Xiaoyan Wu [1], David Kissel [1], Warren S. Rehm [1], Manuel Schwartz [1] and Mumtaz A. Dinno [2]

[1] *Departments of Medicine (Nephrology) and Physics University of Louisville Louisville KY and Department of Physics University of Mississippi Oxford, MS (U S A)*

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Addition of 10^{-5} M amphotericin B to the tear solution of an in vitro preparation of the frog cornea increased the transepithelial conductance, g_t, and decreased the apical membrane fractional resistance, $f(R_0)$, in the presence or absence of tear Na^+ and Cl^-. In the presence of tear Na^+ and Cl^-, amphotericin B increased the short-circuit current, I_{sc}, from 3.9 to 8.8 $\mu A \cdot cm^{-2}$ and changed the intracellular potential, V_0, from −48.5 to −17.9 mV probably due to a higher increase in the Na^+ than in the K^+ conductance. In the absence of tear Na^+ and Cl^-, amphotericin B decreased I_{sc} from 5.5 to about 0 $\mu A \cdot cm^{-2}$ due to K^+ (and possibly Na^+) flux from cell to tear and changed V_0 from −35.4 to −63.6 mV due to the increase in conductance of both ions. Increase in the tear K^+ from 4 to 79 mM (in exchange for choline), in the presence of amphotericin B and absence of tear Na^+ and Cl^-, decreased $f(R_0)$ from 0.09 to 0.06, increased g_t from 0.23 to 0.31 mS, increased I_{sc} from 0.63 to 7.3 $\mu A \cdot cm^{-2}$, and changed V_0 from −65.5 to −17.3 mV due to the change in E_K in the presence of a high conductance in the tear membrane. Similar effects were observed with an increase of tear Na^+. Results support the concept that the Na^+ conductance opened by amphotericin B in the apical membrane is greater than the K^+ conductance. Previously observed transepithelial effects of the ionophore may be explained mostly on the basis of its effect on the apical membrane.

Introduction

Amphotericin B increases the permeability of the apical membrane of the frog cornea epithelium to Na^+ and K^+ [1–4]. That the opened channels by the ionophore are electroconductive is suggested by its effects on transepithelial PD [3,4] and on short-circuit current [1,2]. Further support for the concept that the amphotericin B-opened channels are conductive is that, in the presence of amphotericin B in tear solution, increase in tear concentration of Na^+ or K^+ increases the transepithelial PD, stromal side more positive, and increases the transepithelial conductance, with an opposite effect when the concentration of these cations is decreased [4]. There is evidence that addition of amphotericin B to the tear solution results in an increase in the electrogenicity of the Na^+/K^+-ATPase pump located in the basolateral membrane, i.e., an increase in emf. Thus, the anomalous PD response, which is related to the pump emf, is increased markedly with amphotericin B in Cl^--free solutions when stromal K^+ is increased from 0 to 4 mM [3]. Furthermore, the basolateral K^+ conductance may be affected by changing the tear K^+ concentration in the presence of amphotericin B [4]. The effects on the pump and on the K^+ conductance are probably due to respective changes in intracellular Na^+ and intracellular K^+ concentrations.

The findings described above raise questions which could be answered with the use of intracellular microelectrodes: (1) Which of the two channels, Na^+ and K^+, opened by amphotericin B in the apical membrane has the greater conductance? (2) To what extent are the effects of amphotericin B not only on the tear membrane but also on the stromal membrane? (3) To what extent are the effects of changing tear Na^+ or K^+, in the presence of amphotericin B, on the tear or on both membranes? The results presented below support the concept that the Na^+ channels opened by the ionophore have a greater conductance than the K^+ channels and that the transepithelial effects of the ionophore may be explained mainly on the basis of its effect on the apical membrane.

Correspondence G. Carrasquer, Department of Medicine, Division of Nephrology, University of Louisville, Louisville KY 40292 U.S.A.

Methods

Bullfrog corneas (*Rana catesbeiana*) were mounted tear side up in a lucite chamber as previously described [5–7] The tissue was supported by a copper grid with a slightly less radius of curvature than that of the in vivo cornea An opening of 0 4 cm^2 communicated the upper (epithelial) chamber (0 2 ml) with the lower (stromal) chamber (0 3 ml) Both chambers were continuously perfused at a rate of about 5 ml/min to insure complete exchange in 5–10 s A slight negative hydrostatic pressure was applied to the lower chamber to help secure the cornea to the copper grid Control solutions contained (in mM) Na^+, 102, K^+, 4, Ca^{2+}, 1, Mg^{2+}, 0 8, Cl^-, 81, SO_4^{2-}, 0 8, HCO_3^-, 25, phosphate 1, and glucose 25 Na^+ was substituted with choline$^+$ in Na^+-free solutions Cl^- was substituted with gluconate or SO_4^{2-} in Cl^--free solutions When sulfate was used as the substitute anion sucrose was added in equimolar amounts to maintain a constant osmolality Increases in K^+ concentration were accomplished by substitution of K^+ for choline Amphotericin B was added to the tear solution to a final concentration of 10^{-5} M All solutions were continuously gassed with 95% O_2/5% CO_2, The pH of the solutions was 7 2–7 3 Two pairs of macroelectrodes and one microelectrode were used One pair was used to measure the transepithelial potential difference (calomel electrodes connected via KCl bridges to within 0 5 mm of tissue surfaces), the other pair (AgCl-coated Ag wire loop electrodes, 4 mm from the tissue on either side) was used to send current The intracellular potential, V_0, was recorded with 3 M KCl-filled microelectrodes which had an input resistance of 15–40 Mohm Corneas were short-circuited using an automatic clamp device (Biomed Inst, Germering, F R G) except for brief perturbations that lasted about 200 ms, during which the transepithelial potential was clamped at +10 mV (stroma side positive) These perturbations were repeated every 1–2 s and were used for measurement of the transepithelial conductance ($g_t = \Delta I_t/\Delta V_t$) Also the apical membrane fractional resistance ($f(R_0) = R_0/(R_0 + R_s) = \Delta V_0/\Delta V_t$) could be obtained V_t and I_t are the transepithelial voltage and current, and R_0 and R_s are the resistances across the apical and basolateral membranes, respectively The values of short-circuit current (I_{sc}), g_t, $f(R_0)$, and V_0 were recorded together with the microelectrode resistance on a multichannel strip chart recorder (Linseis, TYP 2065) I_{sc} is defined as positive when the direction of current is from tear to stroma via the tissue Hyperpolarization of V_0 is defined as an increase in the negative intracellular potential Depolarization is used as the opposite of hyperpolarization Student's *t*-test with paired observations was performed to determine the level of significance when applicable

Results

Effect of 10^{-5} M amphotericin B in the tear solution

Figs 1A, 1B and 1C show data from representative experiments in which the ionophore was added to the tear solution The ionophore was added in the presence of control solutions (Fig 1A), in Na^+-free, Cl^--free solutions on tear side and control solutions on stromal side (Fig 1B), and in Na^+-free, Cl^--free tear and stromal solutions (Fig. 1C) Detailed data are presented in Table

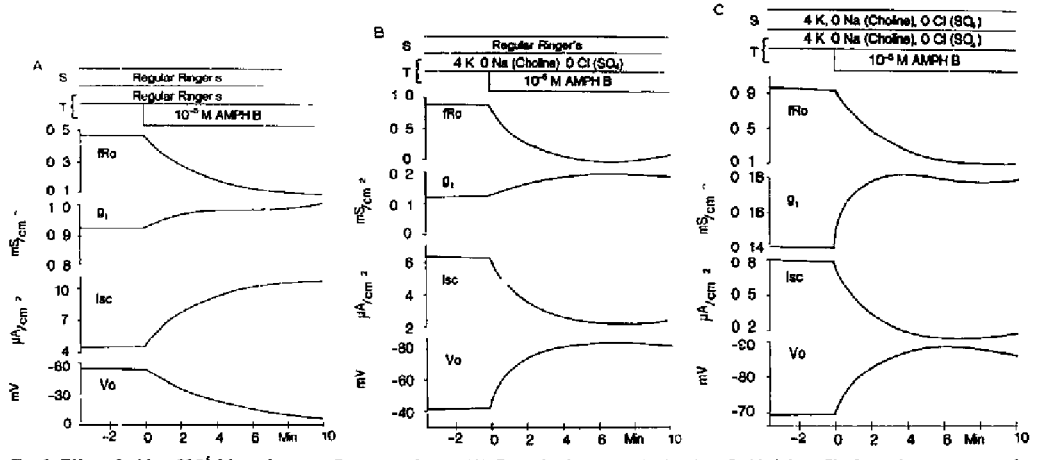

Fig 1 Effect of adding 10^{-5} M amphotericin B to tear solution (A) Control solutions on both sides, (B) Na^+-free, Cl^--free solution on tear side, control solution on stromal side, (C) Na^+-free, Cl^--free solutions on both sides Zero time when amphotericin was added Apical membrane fractional resistance, $f(R_0)$, transepithelial conductance in mS/cm^2, g_t, short circuit current in μA/cm^2, I_{sc}, intracellular potential in mV, V_0

TABLE I

Effect of adding 10^{-5} M amphotericin B to tear solution

Values are mean ± S.E. N = number of experiments. Control values obtained before addition of amphotericin B. The other values are the changes obtained, respectively, 2, 5, and 10 min after addition of the drug. Units are $f(R_0)$ unitless, g_t mS/cm², I_{sc} μA/cm², and V_0 mV. [a] $P < 0.01$ [b] $P < 0.05$ [ns] $P > 0.05$

	Control	Changes in parameter		
		2 min	5 min	10 min
Control solutions ($N = 7$)				
$f(R_0)$	0.29 ± 0.05	0.05 ± 0.03 [ns]	−0.16 ± 3.04 [b]	−0.19 ± 0.05 [b]
g_t	0.78 ± 0.22	0.03 ± 0.009 [b]	0.03 ± 0.009 [b]	0.04 ± 0.[illegible] [b]
I_{sc}	3.9 ± 0.8	1.9 ± 0.5 [a]	3.6 ± 0.8 [a]	3.9 ± 0.7 [a]
V_0	−48.5 ± 7.7	7.5 ± 2.4 [b]	20.2 ± 4.6 [a]	30.7 ± 5.2 [a]
Na^+-free (choline$^+$ subst.), Cl^--free (SO_4^{2-} subst., 8 expts., gluconate subst. 8 expts.) tear soln. control stromal soln. ($N = 14$)				
$f(R_0)$	0.85 ± 0.02	−0.37 ± 0.08 [a]	−0.64 ± 0.05 [a]	−0.73 ± 0.03 [a]
g_t	0.18 ± 0.01	0.04 ± 0.01 [a]	0.06 ± 0.02 [b]	0.08 ± 0.03 [b]
I_{sc}	5.5 ± 0.9	−2.2 ± 0.6 [a]	−4.8 ± 1.3 [a]	−5.4 ± 1.3 [a]
V_0	−35.4 ± 2.4	−13.9 ± 3.7 [a]	−27.8 ± 3.3 [a]	−28.2 ± 3.0 [a]
Na^+-free (choline$^+$ subst.) Cl^--free (SO_4^{2-} subst.) in both soins ($N = 6$)				
$f(R_0)$	0.92 ± 0.03	−0.43 ± 0.11 [a]	−0.65 ± 0.10 [a]	−0.72 ± 0.10 [a]
g_t	0.16 ± 0.02	0.04 ± 0.01 [b]	0.06 ± 0.02 [b]	0.06 ± 0.02 [b]
I_{sc}	1.47 ± 0.44	−0.72 ± 0.19 [b]	−1.03 ± 0.22 [a]	−1.04 ± 0.22 [a]
V_0	−63.70 ± 4.01	−8.53 ± 3.03 [b]	−10.97 ± 1.6[illegible] [b]	−6.3 ± 3.88 [ns]

I. In all cases, amphotericin B decreased markedly the apical membrane fractional resistance, $f(R_0)$, approaching a value of about 0.10 in the presence of Na^+ and of about 0.20 in the absence of Na^+ in both solutions. Amphotericin B increased the transepithelial conductance, g_t, by about 0.04 to 0.08 mS cm^{-2} with or without Na^+ or Cl^-. Moreover, in control solutions (Fig. 1A and Table I), addition of amphotericin B increased the short-circuit current, I_{sc}, and depolarized the intracellular potential, V_0. However, in the absence of Na^+ and Cl^- from tear solution (Fig. 1B and Table I) or from both solutions (Fig. 1C and Table I), amphotericin B gave opposite results in that I_{sc} decreased and V_0 hyperpolarized. The data from experiments of the type presented in Figs. 1A and 1B support the concept that amphotericin B opens a conductive pathway for Na^+ in the apical membrane, since amphotericin B decreased the apical membrane fractional resistance and increased the conductance, also there was an increase in short-circuit current and a depolarization of

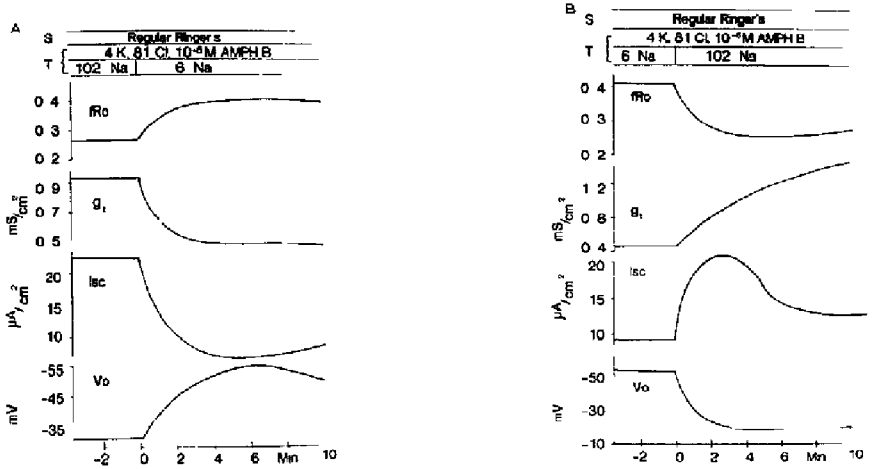

Fig. 2. Effect of changing tear Na^+ in the presence of 10^{-5} M amphotericin B in the tear solution. Meaning of time and symbols as in Fig. 1.

TABLE II

Effects of changing tear Na^+ in presence of 10^{-5} M amphotericin B. Control stromal solution

See legend to Table I. The number of experiments is given in parentheses

	Control	Changes in parameter		
		2 min	5 min	10 min
Decrease tear Na^+ from 102 to 6 mM ($N = 6$)				
$f(R_0)$	0.21 ± 0.06	0.16 ± 0.04 [a]	0.24 ± 0.06 [b]	0.14 ± 0.05 [b]
g_t	1.17 ± 0.12	−0.43 ± 0.09 [a]	−0.54 ± 0.10 [a]	−0.72 ± 0.10 [a]
I_{sc}	18.0 ± 2.6	−11.8 ± 1.5 [a]	−13.2 ± 1.6 [a]	−11.9 ± 1.7 [a]
V_0	−26.8 ± 2.7	−19.0 ± 3.1 [a]	−27.1 ± 4.1 [a]	−29.1 ± 5.0 [a]
Increase tear Na^+ from 6 to 102 mM ($N = 5$)				
$f(R_0)$	0.42 ± 0.05	−0.11 ± 0.02 [a]	−0.14 ± 0.02 [a]	−0.12 ± 0.04 [b]
g_t	0.62 ± 0.14	0.30 ± 0.07 [b]	0.30 ± 0.15 [n.s]	0.47 ± 0.18 [n.s.]
I_{sc}	3.6 ± 2.4	11.9 ± 1.7 [a]	8.8 ± 1.6 [a]	6.9 ± 1.9 [b]
V_t	−52.4 ± 6.8	26.6 ± 5.8 [a]	30.2 ± 6.6 [a]	31.3 ± 6.4 [a]

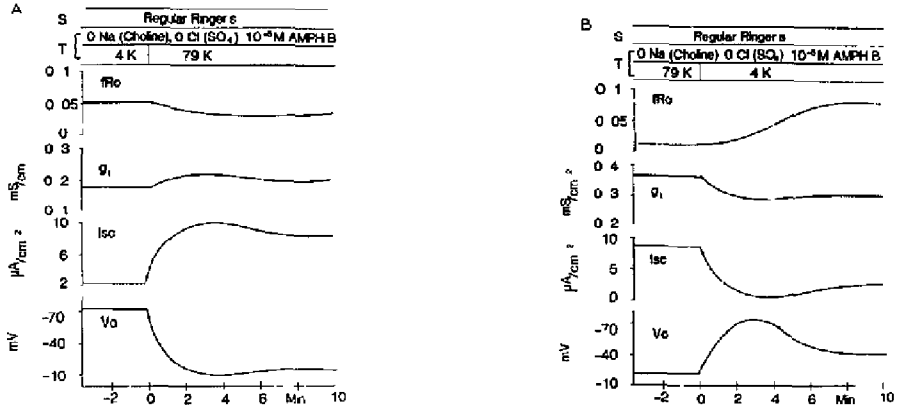

Fig. 3. Effect of changing tear K^+ in the presence of 10^{-5} M amphotericin B in the tear solution. Meaning of time and symbols as in Fig. 1

TABLE III

Effect of changing tear K^+ in tear solution in presence of 10^{-5} M amphotericin B. Control stromal solution

See legend to Table I. The number of experiments is given in parentheses

	Control	Changes in parameter		
		2 min	5 min	10 min
Increase tear K^+ from 4 to 79 mM ($N = 11$)				
$f(R_0)$	0.09 ± 0.02	−0.03 ± 0.007 [a]	−0.03 ± 0.006 [a]	−0.02 ± 0.008 [b]
g_t	0.23 ± 0.03	0.05 ± 0.02 [b]	0.07 ± 0.02 [b]	0.08 ± 0.02 [a]
I_{sc}	0.6 ± 1.4	5.4 ± 1.6 [a]	6.7 ± 1.3 [a]	6.7 ± 1.4 [a]
V_0	−65.6 ± 2.8	37.6 ± 5.7 [a]	47.6 ± 3.4 [a]	48.2 ± 3.2 [a]
Decrease tear K^+ from 79 to 4 mM ($N = 12$)				
$f(R_0)$	0.06 ± 0.02	0.05 ± 0.02 [b]	0.05 ± 0.02 [b]	0.04 ± 0.01 [b]
g_t	0.33 ± 0.05	−0.07 ± 0.03 [b]	−0.08 ± 0.03 [b]	−0.08 ± 0.03 [b]
I_{sc}	7.9 ± 1.0	−7.1 ± 1.2 [a]	−7.1 ± 1.1 [a]	−6.3 ± 1.1 [a]
V_0	−16.3 ± 2.3	−42.4 ± 2.3 [a]	−39.0 ± 2.7 [a]	−31.8 ± 4.1 [a]

the intracellular potential when the electrochemical potential of Na^+ was higher in the tear solution than in the cell (presence of 102 mM Na^+ in tear) and a decrease in short-circuit current and hyperpolarization of the intracellular potential when the electrochemical potential of Na^+ was higher in the cell than in the tear solution (Na^+-free, Cl^--free tear solution) Data from experiments of the type presented in Fig 1C support the concept that amphotericin B opens a conductive pathway for K^+ since this ion should be more abundant than Na^+ in the cell under conditions of Na^+-free, Cl^--free in both solutions and the decrease in I_{sc} and hyperpolarization could be explained by the presence of an apical membrane K^+ emf and by net K^+ flux from cell to tear The decrease in I_{sc} and the hyperpolarization of V_0 suggest that there is still a high K^+ in the cell in the absence of Na^+ and Cl^- in the media

Effect of changing tear Na^+ in the presence of amphotericin B in tear solution

Data presented in Fig 2 from one representative experiment and in Table II further support the concept that amphotericin B opens conductive channels to Na^+, that is, decrease in tear Na^+ from 102 to 6 mM (Fig 2A and Table II) in the presence of amphotericin B increased $f(R_0)$, decreased g_t and I_{sc}, and hyperpolarized V_0 Increase in tear Na^+ back to 102 mM produced the opposite results (Fig. 2B and Table II)

Effect of changing tear K^+ in the presence of amphotericin B in tear in Na^+-free and Cl^+-free solutions

Data presented in Fig 3 from a representative experiment and in Table III support the concept that amphotericin B opens conductive channels to K^+, that is, increase in tear K^+ from 4 to 79 mM (Fig 3A and Table III) decreased $f(R_0)$, increased g_t and I_{sc}, and depolarized V_0 Decrease in tear K^+ back to 4 mM (Fig 3B and Table III) produced changes opposite to those observed when tear K^+ was increased from 4 to 79 mM

Discussion

Previous experiments indicate that the permeability of the apical membrane to Na^+ and K^+ is markedly increased by amphotericin B [1–4] The possibility that the ionophore-opened pathways were conductive was considered when addition of amphotericin B increased the short-circuit current and the transepithelial conductance [1,2] and when changes in tear Na^+ and K^+ concentration resulted in transepithelial PD changes [3,4]

A question was raised whether the changes observed with amphotericin B depended upon an effect on the basolateral membrane as well as on the apical membrane For example, increase of Na^+ entrance into the cell could stimulate the electrogenic Na^+/K^+-ATPase [7 8] with a subsequent hyperpolarization of the basolateral membrane and an increase in V_0 On the other hand, with a low tear K^+ the intracellular K^+ should decrease because of a leakage of this ion across the apical membrane with a subsequent decrease in the potential across the basolateral membrane Similarly, in the presence of amphotericin B, when Na^+ or K^+ is decreased or increased in the tear solution, one would expect parallel intracellular changes in concentration of these ions Hence an increase in tear Na^+ or K^+ should result in an increase in intracellular Na^+ or K^+ with an increase in the cell to stromal Na^+ or K^+ concentration ratio, therefore, an increase in the negativity of the cell with respect to stroma, that is, hyperpolarization of the potential across the basolateral membrane With a Na^+ or K^+ conductive pathway in the apical membrane, the effects on the potential between the cell and the tear solution should be opposite to those in the basolateral membrane, that is, an increase in tear Na^+ or K^+ decreases the ratio of cell to tear Na^+ or K^+ concentration with a depolarization or even reversal of the potential across the apical membrane

For further discussion we refer to the relationship

$$V_{ST} = V_{SC} - V_{TC} \qquad (1)$$

where V_{ST}, V_{SC}, and V_{TC} are the potentials between stromal and tear solutions, between stroma and cell, and between tear and cell respectively Both, depolarization of the apical membrane potential (decrease in V_{TC}) and hyperpolarization of the basolateral membrane potential (increase in V_{SC}) due to an increase in the tear Na^+ or K^+ will increase the transepithelial potential V_{ST}, stromal side more positive, previously observed under open-circuit conditions in the presence of amphotericin B [4]

Present experiments using microelectrodes, under short-circuit conditions, demonstrate that the effect of amphotericin B is primarily on the apical membrane, although a secondary effect of lesser magnitude in the basolateral membrane cannot be ruled out (1) Under all conditions studied, upon addition of amphotericin B or after an increase in the tear Na^+ or K^+ in the presence of the ionophore, there was an increase in the transepithelial conductance which was accompanied by a decrease in the apical membrane fractional resistance (2) Under conditions in which the tear concentration of Na^+ (102 mM) was higher than in the cell amphotericin B increased the short circuit current and presumably the open circuit transepithelial potential (the calculated $V_{ST} - I_{sc}/g_t$ increased) and they were accompanied by a depolarization (decrease in negativity of V_0), that is, the increase in V_{ST} was primarily due to a decrease in V_{TC} (see Eqn 1) (3) The effects of changing the tear Na^+ in the presence of amphotericin B further corroborate the opening of a Na^+ conductance in the apical membrane,

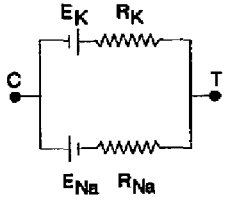

Fig 4 Equivalent circuit for Na^+ and K^+ pathways opened by amphotericin B in the apical membrane of the frog cornea epithelium T refers to tear and C refers to cell See text for symbols

that is, an increase in tear Na^+ increased the transepithelial conductance which was accompanied by a decrease in the apical membrane fractional resistance This increase in Na^+ concentration enhanced the short-circuit current as a result of a depolarization of the apical membrane (decreased negativity of V_0), therefore, the increase in calculated V_{ST} was due to a decrease of V_{TC} (see Eqn 1) (4) The opening of an apical membrane K^+ conductance was demonstrated when the tear K^+ concentration was changed in the absence of Na^+ and Cl^- from the tear solution and in the presence of amphotericin B Increase in tear K^+ resulted in the expected increase in transepithelial conductance, decrease in the apical membrane fractional resistance, increase in short-circuit current (increase also in calculated V_{ST}) with depolarization of V_0, that is, decrease in V_{TC}

We now consider the question whether with amphotericin B present Na^+ or K^+ has the greater conductance in the apical membrane The possible contribution of the two pathways to the effects of amphotericin B may be evaluated by an analysis of the circuit presented in Fig. 4 For this circuit the following equation applies

$$V_{TC} = E_K R_{Na}/(R_{Na} + R_K) - E_{Na} R_K/(R_{Na} + R_K) \quad (2)$$

where R's are the resistances of the opened Na^+ and K^+ pathways by amphotericin B in the apical membrane and E's are the respective emf's If we assume that intracellular K^+ and Na^+ are, respectively, 106 and 14 mM, as reported by Reuss et al [9], E_K should be somewhat greater than E_{Na} i e $(RT/F)\ln(106/4) > (RT/F)\ln(102/14)$ Therefore, with equal resistance values for Na^+ and K^+, V_{TC} should have increased when amphotericin B was added to the tear solution Since it decreased, that is, since the cell became less negative to the tear solution, R_K must be greater than R_{Na}, that is, the Na^+ conductance opened by amphotericin B must be higher than the opened K^+ conductance

Not considered in this work is the possible effect of amphotericin B on the Cl^- conductance, but, since the tear Cl^- concentration was higher than its intracellular concentration (81 versus 22 mM [9]), an increase in the Cl^- conductance by amphotericin B should have increased V_{TC} (hyperpolarization) Therefore, the depolarization observed in the presence of Na^+ and Cl^- cannot be explained by an increase in the Cl^- conductance by amphotericin B As in the case of Na^+ versus K^+, one may safely say that if amphotericin B increased the Cl^- conductance, its increase was of a lesser magnitude than the increase in the Na^+ conductance

In conclusion, the pathways opened to Na^+ and K^+ by amphotericin B in the apical membrane of the frog cornea epithelium are conductive The Na^+ conductance appears to be greater than the K^+ conductance Possible effects of amphotericin B on the Cl^- conductance need further study Transepithelial effects of the ionophore may be explained mostly on the basis of its effect on the apical membrane

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