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Effect of Ba^{2+} on the K^+ conductance pathways in the frog cornea

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Two types of transepithelial potential difference (PD) responses have been observed in the bullfrog, *Rana catesbeiana*, when the K^+ concentration is changed in the aqueous solution. (1) A normal response, that is, a decrease in the positivity of the aqueous solution when the K^+ is increased in this solution. (2) An anomalous response, that is, an increase in PD when K^+ is increased from 0 to 4 mM in the aqueous solution. In present experiments 2 mM Ba^{2+} results in a significant decrease in transepithelial PD and an increase in resistance (*R*), consistent with the well-known effect of Ba^{2+} on the K^+ conductance in other biological membranes. In the presence of Ba^{2+} compared to its absence the normal PD responses were decreased when K^+ was increased from 4 to 20 or to 79 mM in the aqueous solution. Barium enhanced, but not significantly, the anomalous PD response (PD increase) when K^+ was increased from 0 to 4 mM. An anomalous PD response (PD decrease) was obtained with Ba^{2+} when K^+ was changed from 4 to 0 mM while in its absence the response was normal (PD increase) or did not change. These findings support the concept that anomalous PD responses as a result of the electrogenic $(Na^+ + K^+)$-ATPase may be obtained when the resistance of the simple K^+ pathway is increased.

Introduction

Using the ion pulsing technique, we have found two types of PD responses to changes in K^+ concentration in the solution bathing the stromal or aqueous side of the frog cornea [1]. First, an increase in K^+ in the aqueous solution results in a decrease in positivity of the stroma relative to the tear side. We refer to this as a normal response, since it is what one would expect if a simple conductance pathway (K^+ pathway) exists in the basolateral membrane of the epithelial cells. The second type is obtained after bathing the cornea in K^+-free solutions, when the aqueous K^+ is increased from 0 to 4 mM, which results in an increase in the transcellular PD; the stroma side becomes more positive relative to the tear side. We call this second type an anomalous PD response since it is contrary to what one would expect from a simple K^+ conductance pathway. We have attributed the anomalous PD response to the presence of an electrogenic $(Na^+ + K^+)$ATPase pump (pump pathway) in the basolateral membrane transporting more Na^+ than K^+ ions per cycle. The presence of the simple K^+ pathway is well supported [1–4]. Candia et al. [5] have data using fluxes that support the electrogenicity of the $(Na^+ + K^+)$-ATPase pump.

Our working model is a K^+ pathway and a pump pathway in parallel. Each pathway can be represented by a resistance and an e.m.f. Usually, the resistance of the K^+ pathway is much lower than the resistance of the pump pathway. Therefore the K^+ pathway predominates and normal PD responses are obtained when the K^+ con-

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Abbreviations: PD, potential difference; *R*, resistance.

centration is changed in the aqueous solution. With zero K^+ in bathing solutions, the K^+ pathway resistance increases markedly leaving the pump as the predominant K^+ pathway and therefore results in anomalous PD responses.

To test this hypothesis further we designed experiments changing the K^+ concentration in the aqueous solution in the presence of Ba^{2+}. Ba^{2+} is well known to increase the K^+ resistance pathway of various epithelia [6–10]. We will show in this paper that the normal PD responses to changes in the K^+ concentration in the aqueous solution are reduced and the anomalous PD responses enhanced with Ba^{2+} in the aqueous solution.

Methods

Experiments were performed on corneas of the bullfrog (*Rana catesbeiana*) by an in vitro method in which the corneas were mounted between a pair of cylindrical chambers [1,2,11].

All experiments were begun with physiological (control) solutions on both sides of the cornea. 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 10. Na^+ was substituted for K^+ in K^+-free solutions. K^+ was increased in substitution for Na^+. $BaCl_2$ was added to the aqueous solution to a final concentration of $2 \cdot 10^{-3}$ M.

Both sides of the cornea were continually gassed with 95% O_2/5% CO_2. Gassing was used also to recirculate and stir thoroughly the solutions. The pH of the solution was 7.2–7.3.

To avoid transmembrane changes in pressure when changing the solutions, the chambers were never drained out. The new solution was injected at a high flow rate (10- to 15-times the volume of the chamber per minute) from the bottom of the chamber. The outflow was located on the top of the chamber. The outflow tube of the aqueous solution was located about 15 cm in about one-third of the experiments and 2 cm in about two-thirds of the experiments, above the outflow tube of the tear solution. The 15 cm pressure difference was used because of its similarity to the in vivo intraocular pressure. The 2 cm pressure difference was sufficient to preserve the natural shape of the cornea. The electrical parameters were not affected by a change from one pressure gradient to the other.

The transepithelial resistance (R) and transepithelial electrical potential (PD) were measured. Two pairs of electrodes were used: one for sending current across the mucosa and the other for measuring the PD. The PD was taken as positive when the aqueous side was positive to the tear side. The resistance was determined as the change in PD per unit of applied current. Two microamperes were applied for 1 or 2 s in one direction and 2 to 10 s later in the other direction. No significant rectification was observed. Student's *t*-test using paired observations was performed to determine the level of significance, when applicable.

Results

Effect of Ba^{2+} on PD and resistance

Fig. 1 shows that addition of 2 mM Ba^{2+} in the aqueous solution results in a decrease in PD and an increase in resistance.

In 12 experiments the PD decreased significantly by 6.2 (S.E. $\pm$ 0.8) from 15.2 mV (S.E. $\pm$ 1.719) and the resistance increased significantly by 0.52 (S.E. $\pm$ 0.10) from 1.87 (S.E. $\pm$ 0.16) kohm $\cdot$ cm^2.

However, 2 mM Ba^{2+} in the tear solution in six experiments did not affect the transepithelial PD or resistance, indicating a low partial conductance to K^+ in the apical membrane.

Effect of 2 mM Ba^{2+} in the aqueous solution on the PD and R responses to changes in K^+ concentration in the aqueous solution in control conditions

Fig. 1 shows a typical experiment with PD and resistance responses to changes in K^+ from 4 to 79 mM in the aqueous solution in the absence and presence of Ba^{2+}. The change in PD was smaller with Ba^{2+} in the aqueous solution than the change before Ba^{2+} was added or after its removal from the solution. As shown in Fig. 1, the K^+ concentration was increased from 4 to 79 mM in both tear and aqueous solutions simultaneously and the change in PD was essentially the same as with the increase in K^+ in the aqueous solution alone. The decrease in resistance observed when the K^+ concentration was increased from 4 to 79 mM was greater in the presence of 2 mM Ba^{2+} than before or after Ba^{2+}.

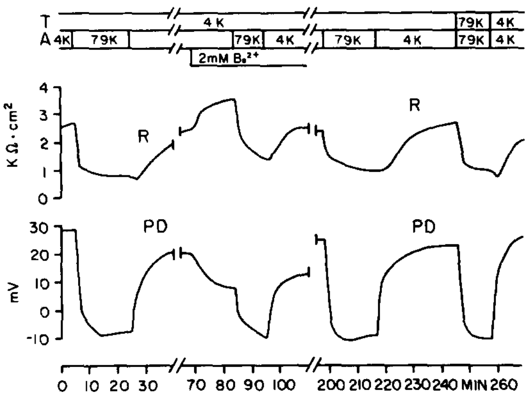

Fig. 1. Effect of Ba^{2+} on the normal PD responses to changes in K^+ concentration in the aqueous solution.

Table I shows the effects of changing aqueous K^+ in the presence and absence of Ba^{2+} as illustrated in the first part of Fig. 1. Decreases in PD were significantly smaller ($P < 0.05$) and increases in resistance significantly greater ($P < 0.05$) in the presence of Ba^{2+} than in its absence. The effect of Ba^{2+} on the response to changes in K^+ concentration was similar for changes from 4 to 20 and from 4 to 79 mM K^+. Ten minutes following the change in aqueous K^+ from 4 to 20 mM, the PD was 3.0 (S.E. ± 1.5) mV in the absence of Ba^{2+} and 3.1 (S.E. ± 0.9) mV in the presence of Ba^{2+}. Similarly, 10 min following the change in aqueous K^+ from 4 to 79 mM, the PD was −9.7 (S.E. ± 0.4) mV in the absence of Ba^{2+} and −9.3 (S.E. ± 1.8) in its presence. Thus, while the magnitude of the change in PD was smaller in the presence than in the absence of Ba^{2+} when the aqueous concentration of K^+ was increased, the PD level reached with the increase in K^+ was about the same whether Ba^{2+} was present or not. On the other hand, when the K^+ concentration was brought back to 4 mM, either from 20 or 79 mM, both the PD and the change in PD 10 min after the change in concentration were smaller in the presence of Ba^{2+} than in its absence. The increase in R when K^+ was decreased from 20 or 79 to 4 mM was not significantly different in the presence or absence of Ba^2.

Effect of 2 mM Ba^{2+} in the aqueous solution on PD and R responses to change in K^+ concentration to and from zero K^+ in the aqueous solution

Fig. 2 shows data on PD and R versus time from a typical experiment in which K^+ was removed from and then returned to 4 mM in the aqueous solution with and without 2 mM Ba^{2+} in that solution. When K^+ was increased from zero to 4 mM the typical [1] anomalous increase in PD was observed. In the presence of Ba^{2+} not only an anomalous PD response was obtained when changing from zero to 4 mM as expected but also an anomalous (decrease) PD was observed when the K^+ was decreased from 4 to 0 mM.

Data presented in Table II show that, in the absence of Ba^{2+}, with the removal of K^+ from the

TABLE I

EFFECT OF CHANGING K^+ CONCENTRATION IN THE AQUEOUS SOLUTION IN CONTROL AND IN THE PRESENCE OF 2 mM Ba^{2+}

Values are means ± S.E. PD and R control values are obtained before the exchange. PD and R expt. values are obtained 10 min after the change. ΔPD = Expt. − Control. ΔR = Expt. − Control.

Aqueous solution		No. of expts.	PD (mV)		ΔPD (mV)	R (kohm·cm²)		ΔR (kohm·cm²)
Orig. (mM)	Final (mM)		Control	Expt.		Control	Expt.	
4 K	20 K	7	13.1 ± 2.3	3.0 ± 1.5	−10.1 ± 1.0 *	1.23 ± 0.15	1.06 ± 0.14	−0.17 ± 0.03 *
4 K	79 K	6	22.6 ± 2.6	−9.7 ± 0.4	−32.3 ± 2.9 *	1.46 ± 0.08	0.78 ± 0.03	−0.68 ± 0.08 *
2 mM Ba^{2+} in aqueous solution								
4 K	20 K	7	6.4 ± 1.2	3.1 ± 0.9	−3.4 ± 0.4 *	2.02 ± 0.31	1.53 ± 0.24	−0.49 ± 0.09 *
4 K	79 K	6	9.7 ± 1.4	−9.3 ± 1.8	−19.1 ± 2.8 *	2.73 ± 0.33	1.13 ± 0.14	−1.60 ± 0.23 *

* $p < 0.01$.

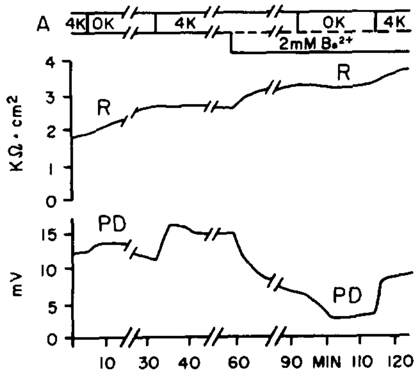

Fig 2. Effect of Ba^{2+} on the anomalous PD responses to changes in K^+ concentration to and from 0 mM in the aqueous solution.

aqueous solution the PD increased significantly to a maximum (within about 2 min) and then decreased. Ten minutes later the PD was not significantly different from control. The increase in *R* was significant both at the level of maximum PD and 10 min after removal of K^+.

Upon return of the K^+ concentration to 4 mM, in the absence of Ba^{2+}, the typical [1] anomalous increase in PD was observed. The maximum increase was observed between 2 and 5 min after increasing the K^+ concentration, and the PD remained significantly higher than the control 10 min later. The resistance decreased significantly throughout.

In the presence of 2 mM Ba^{2+} in the aqueous solution, removal of K^+ from this solution resulted, as indicated above, not in a normal PD response (PD increase) but in an anomalous response (PD decrease), as illustrated in Fig. 2 at about 95 min. There was no significant change in resistance. This finding on PD was different from that in the absence of Ba^{2+}. The difference is obviously due to a marked decrease of the K^+ conductance by Ba^{2+} (see Discussion).

When K^+ was increased from zero to 4 mM in the presence of Ba^{2+}, an anomalous increase in PD was observed similar to that observed in the absence of Ba^{2+}. On the other hand the resistance did not change significantly contrary to the significant decrease observed in the absence of Ba^{2+}. Again this can be explained on the basis of the effect of Ba^{2+} on the simple K^+ conductance pathway (see Discussion).

Discussion

The presence of two K^+ conductive pathways in the basolateral membrane of the cornea epithelium, a simple K^+ conductive pathway [1–4] and an electrogenic ($Na^+ + K^+$)-ATPase pathway [1,5] are further supported by present experiments.

Barium is well known to decrease the conductance of the simple K^+ pathway in other biological membranes [6–10]. Its effect on the cornea epi-

TABLE II

EFFECT OF CHANGING K^+ CONCENTRATION IN THE AQUEOUS SOLUTION IN CONTROL AND IN THE PRESENCE OF 2 mM Ba^{2+}

Values are means ± S.E. PD and *R* control values are obtained before change. Δ(max) values are obtained at the highest PD following change (< 10 min). Δ(10 min) are values obtained 10 min after change.

Aqueous solution		No. of expts.	PD (mV)	ΔPD(max) (mV)	ΔPD(10 min) (mV)	*R* (kohm·cm²)	ΔR (at max PD) (kohm·cm²)	ΔR (10 min) (kohm·cm²)
Orig. (mM)	Final (mM)							
4 K	0 K	12	15.2 ± 2.8	3.2 ± 0.5 *	1.1 ± 1.0	1.74 ± 0.10	0.23 ± 0.04 *	0.48 ± 0.12 *
0 K	4 K	12	13.4 ± 2.2	7.3 ± 0.9 *	4.4 ± 0.7 *	2.14 ± 0.18	−0.24 ± 0.06 *	−0.32 ± 0.09 *
2 mM Ba^{2+} in aqueous solution								
4 K	0 K	7	7.9 ± 0.9	–	−3.9 ± 0.7 *	2.00 ± 0.28	–	0.05 ± 0.05
0 K	4 K	7	5.8 ± 1.3	7.3 ± 1.1 *	4.1 ± 0.8 *	2.12 ± 0.33	−0.02 ± 0.10	0.01 ± 0.14

* $p < 0.01$.

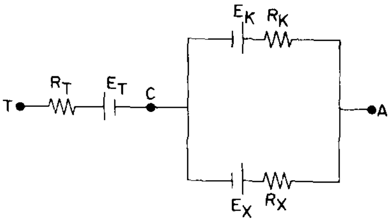

Fig. 3. Equivalent circuit for conductive pathways in the apical and basolateral membranes of frog cornea epithelium. See text for description.

thelium may be similarly explained. Fig. 3 presents a circuit, for which

$$R = R_T + (R_K R_X)/(R_K + R_X) \quad (1)$$

$$PD_{AT} = PD_{AC} + PD_{CT} = -E_T + E_X + R_X(E_K - E_X)/(R_X + R_K) \quad (2)$$

where R is the transepithelial resistance; R_T is the apical membrane resistance; and R_K and R_X are the resistances in the K and X pathways in the basolateral membrane. The X pathway represents all conductive pathways other than K. To simplify our analysis we have assumed that the paracellular resistance is very large and for ease of illustration will be ignored. PD_{AT} is the transepithelial potential difference; PD_{AC} and PD_{CT} are the PDs across the basolateral and apical membranes, respectively. E_T is the equivalent circuit e.m.f. across the apical membrane; and E_K and E_X are the e.m.f.s of the K and X pathways in the basolateral membrane.

Barium added to the aqueous solution resulted in an increase in R and a decrease in PD. As in other biological membranes these findings may be explained on the basis of an increase in R_K. The increase in the transepithelial resistance could be due to an increase in R_T, R_K or R_X. Since the addition of Ba^{2+} to the tear solution did not affect the resistance or PD, it is unlikely that the transepithelial increase in R is due to an increase in R_T. It also indicates that Ba^{2+} does not change the resistance of the paracellular pathway. The fact that the increase in R by Ba^{2+} was quickly reversed by high concentrations of K^+ (20 or 79 mM) in the aqueous solution shows that Ba^{2+} blocks the K^+ channels. Furthermore, if we consider the thickness of the stroma, the reversal is essentially instantaneous [7].

The decrease in PD by Ba^{2+} can also be explained by its effect on R_K, that is, the K^+ resistance in the basolateral membrane. From Eqn. 2 an increase in R_K would result in a decrease in the third term on the right side of the equation and a decrease in PD_{AT}. For PD_{AT} to decrease when R_K increases, E_K must be greater than E_X.

To understand the effect of Ba^{2+} on the PD responses to changes in K^+ concentration we use the circuit as shown in Fig. 4, for which

$$\Delta PD = (R_K/[R_P + R_K])\Delta E_P - (R_P[R_P + R_K])\Delta E_K \quad (3)$$

where R_K and R_P are the resistances of the simple K^+ conductive and electrogenic $(Na^+ + K^+)$-ATPase pump pathways, respectively; ΔE_P and ΔE_K are the changes in the pump and simple K^+ pathway e.m.f.s when the K^+ concentration is increased in the aqueous solution. A normal ΔPD response (PD decrease) will occur if the term with E_K predominates, while an anomalous (PD) increase) response will take place if the term with E_P predominates. With 4 mM K^+ in the aqueous solution, normal responses were observed when the K^+ concentration was increased to 20 or 79 mM. With an increase in R_K by Ba^{2+} one may explain why ΔPD was smaller in the presence of Ba^{2+} than in its absence (see Fig. 1 and Table I), that is, the coefficient of $(E_K - E_X)$ would be smaller in the presence of Ba^{2+} than in its absence. The fact that, although smaller, the PD response was normal in the presence of Ba^{2+} shows that R_K was rapidly decreased by 20 or 79 mM K^+ in the presence of Ba^{2+} (see Eqn. 2). It is to be noted that the magnitude of the PD with high K^+ concentra-

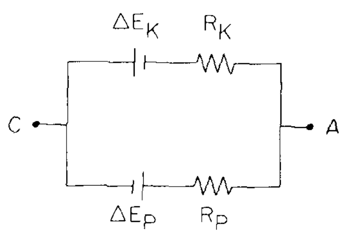

Fig. 4. Equivalent circuit for K^+ pathways in basolateral membrane of frog cornea epithelium. Simple conductive (K^+) and $(Na^+ + K^+)$-ATPase pump (P) pathways. For a K^+ concentration increase in aqueous solution, the orientation of ΔE_K and ΔE_P are as shown in figure, where ΔE_K refers to change in e.m.f. C refers to cell and A refers to aqueous (or stroma).

tions in the aqueous solution was about the same with or without Ba^{2+} ($P > 0.8$). This supports the concept that high K^+ abolished the Ba^{2+} effect.

The anomalous response obtained when the K^+ concentration was changed from 0 to 4 mM was not significantly different in the presence or absence of Ba^{2+}. Apparently R_K is so high with 0 mM K^+, that Ba^{2+} did not influence the response. On the other hand, when the K^+ concentration was decreased from 4 to 0 mM an anomalous (PD decrease) change in PD was obtained only in the presence of Ba^{2+}, that is, 4 mM K^+ was not sufficient to counteract the effect of Ba^{2+} on the simple K^+ conductive pathway.

In conclusion, decreases in PD and increases in resistance by 2 mM Ba^{2+} in the aqueous solution support the concept that Ba^{2+} decreases the simple K^+ conductive pathway in the basolateral membrane of the cornea epithelium. The increase in R_K results in smaller normal PD responses to increases in K^+ concentration in the aqueous solution. Likewise, the increase in R_K by Ba^{2+} evokes an anomalous PD response when K^+ is decreased from 4 to 0 mM, an effect not observed in the absence of Ba^{2+}.

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References

1 Carrasquer, G., Ahn, S., Schwartz, M. and Rehm, W.S. (1985) Am. J. Physiol., in the press
2 Graves, C.N., Sanders, S.S., Shoemaker, R.L. and Rehm, W.S. (1975) Biochim. Biophys. Acta 389, 550–556
3 Reuss, L., Reinach, P., Weinman, S.A. and Grady, T.P. (1983) Am. J. Physiol. 244, C336–C347
4 Reinach, P. and Nagel, W. (1984) Fed. Proc. 43, 893
5 Candia, O.A., Reinach, P.S. and Alvarez, L. (1984) Am. J. Physiol. 247, C454–C461
6 Schwartz, M., Pacifico, A.D., MacKrell, T.N., Jacobson, A. and Rehm, W.S. (1968) Proc. Soc. Exp. Biol. Med. 127, 223–225
7 Pacifico, A.D., Schwartz, M., MacKrell, T.N., Spangler, S.G., Sanders, S. and Rehm, W.S. (1969) Am. J. Physiol. 216, 536–541
8 Nagel, W. (1979) Biochim. Biophys. Acta 552, 346–357
9 Biagi, B., Sohtell, M. and Giebisch, G. (1981) Am. J. Physiol. 241, F677–686
10 Lau, K.R., Hudson, R.L. and Schultz, S.G. (1984) Proc. Natl. Acad. Sci. USA 81, 3591–3594
11 Candia, O.A. (1972) Am. J. Physiol. 223, 1053–1057