Biochimica et Biophysica Acta 900 (1987) 258–266
Elsevier

BBA 73581

Microelectrode studies of potential difference responses to changes in stromal K^+ in bullfrog cornea

Gaspar Carrasquer [a], Wolfram Nagel [b], Warren S. Rehm [a] and Manuel Schwartz [a]

[a] *Departments of Medicine (Nephrology) and Physics, University of Louisville, Louisville, KY 40292 (U.S.A.) and* [b] *Department of Physiology, University of Munich, Munich (F.R.G.)*

(Received 12 March 1987)

Key words: ATPase, $(Na^+ + K^+)$-; Potassium-free solution; Intracellular potential; Short-circuit current; Voltage clamp technique; Microelectrode technique; Ion pulse technique; (Bullfrog cornea)

The effects of changing stromal K^+ were studied using microelectrodes in an in vitro preparation of frog cornea. The intracellular potential (V_0) responded in two opposite ways under short-circuit conditions: (1) depolarization (normal response) when stromal K^+ was increased from 4 to 20 or to 79 mM, about 30 mV per 10-fold K^+ concn. change; (2) a hyperpolarization (anomalous response) of 10 mV maximum when stromal K^+ was increased from 0 to 4 mM. The increase from 4 to 20 or 79 mM decreased or even reversed the short-circuit current (I_{sc}). The transepithelial conductance (g_t) increased when K^+ was increased to 79 mM but no change occurred in the apical membrane fractional resistance (fR_o). Increase of stromal K^+ from 0 to 4 mM increased I_{sc} and minimally changed g_t and fR_o. Ouabain (10^{-3} M) abolished the anomalous responses, that is, the increases in V_0 and I_{sc} when stromal K^+ was increased from 0 to 4 mM. These results are interpreted in terms of two K^+ conductive pathways in the basolateral membrane of the corneal epithelium, a Nernstian conductance and an electrogenic $(Na^+ + K^+)$-ATPase pump transporting more Na^+ than K^+ ions per cycle. The normal or anomalous potential difference responses to changes in stromal K^+ appear to depend on the relative resistance of the two pathways at the time stromal K^+ is changed.

Introduction

The corneal epithelium can actively transport Cl^- from stroma to the tear side [1] and to a lesser extent Na^+ from the tear to the stroma side [2]. Under conditions of increased apical membrane permeability, i.e., in the presence of amphotericin B in the tear solution, the transport of Na^+ can be markedly increased and active transport of K^+ from stroma to tear may be also observed [3]. The primary transport mechanism appears to be a $(Na^+ + K^+)$-ATPase pump, located in the basolateral membrane of the epithelial cells facing the stroma, which is directly responsible for the transport of Na^+ and K^+. A Na^+/Cl^- symport [4,5], located also in the basolateral membrane, together with the $(Na^+ + K^+)$-ATPase pump, appears to be responsible for the active transport of Cl^-. As a result of the $(Na^+ + K^+)$-ATPase pump the intracellular Na^+ concentration is maintained low inducing a forced ion-coupled diffusion of Na^+ and Cl^- with Cl^- moving against its electrochemical potential gradient [4], therefore, achieving the high intracellular electrochemical potential of Cl^-

Correspondence: G. Carrasquer, Department of Medicine (Nephrology), Health Sciences Center, University of Louisville, Louisville, KY 40292, U.S.A.

[6]. The $(Na^+ + K^+)$-ATPase pump is sensitive to ouabain and vanadate [7,8] and transports more Na^+ than K^+ ions per cycle, that is, the pump is electrogenic [3,8]. The basolateral membrane has a conductive pathway for K^+ [5,8].

Using the ion substitution method, we have demonstrated the presence of the K^+ simple conductive pathway and the electrogenicity of the $(Na^+ + K^+)$-ATPase pump [8]. The electrogenicity of the pump may be enhanced by addition of amphotericin B to the tear solution in the presence of Cl^--free (SO_4^{2-} substituted) solutions [9]. The simple K^+ conductive pathway may be inhibited by the presence of Ba^{2+} in the stromal solution [10]. The response of the emf of the pump to changes in K^+ in the stromal solution is opposite to the response of the simple conductive pathway located in the same membrane as previously obtained in frog gastric mucosa [11]. The demonstration of the electrogenicity of the pump was accomplished by adding 4 mM K^+ to the stromal solution after bathing the cornea in K^+-free solution for 5–10 min. Presumably the resistance of the simple K^+-conductive pathway increased in K^+-free solutions to the point that, with an increase in K^+ from 0 to 4 in the stromal solution, the emf of the pump dominated, with an increase in potential difference (PD) (stroma more positive) which was opposite to the change expected from a simple conductive pathway. This was designated as an anomalous response, in contrast to the usual normal response, i.e., a decrease in PD with an increase in K^+ concentration [8].

In the above mentioned studies, PD and resistance were recorded across the entire corneal epithelium and responses to changes in stromal solution were assumed to be mostly due to PD and resistance changes across the basolateral membrane, since the cornea is a tight, high resistance epithelium. To test the verity of the assumption, experiments were performed using microelectrodes and recording the effects on PD and resistance due to changes in stromal K^+, not only across the entire epithelium, but also across the apical and basolateral membranes.

Methods

Bullfrog corneas (*Rana catesbeiana*) were mounted tear side up in a lucite chamber as previously described [12,13]. 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^+ 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^+. Ouabain was added to stromal solution to a final concentration of 10^{-3} 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 to obtain the data. 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 was recorded with 3 M KCl-filled microelectrodes which had an input resistance of 15–40 Mohm. In most experiments the 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 ($fR_o = R_o/(R_o + R_i) = \Delta V_o/\Delta V_t$) could be obtained. V_t and I_t are the transepithelial voltage and current, and R_o and R_i are the resistances across the apical and basolateral membranes, respectively. The values of short-circuit current (I_{sc}), g_t, fR_o, and V_o were recorded together with the microelectrode resistance on a multichannel strip chart recorder (Servogor, BBC-Mellard). I_{sc} is defined as positive when the direction of current is from tear to stroma via the tissue. The values of the open-circuit transep-

ithelial potential were calculated from $V_t = I_{sc}/g_t$. These values were checked and confirmed experimentally by briefly opening the circuit and actually measuring the open circuit potential difference. In open circuit experiments I_{sc} was held down at zero and, in its place, V_t was continuously recorded. V_o was used as the symbol for transapical membrane potential and V_i for the transbasolateral membrane potential. V_t, V_i and V_o are defined respectively as the potentials of stroma relative to tear, of stroma relative to cell and of tear relative to cell. That is $V_t = V_i - V_o$. Hyperpolarization of V_o or V_i is defined as an increase in their positive value. Depolarization is used as the opposite of hyperpolarization. All other parameters were recorded as in the short-circuit experiments. Student's t-test with paired observations was performed to determine the level of significance, when applicable.

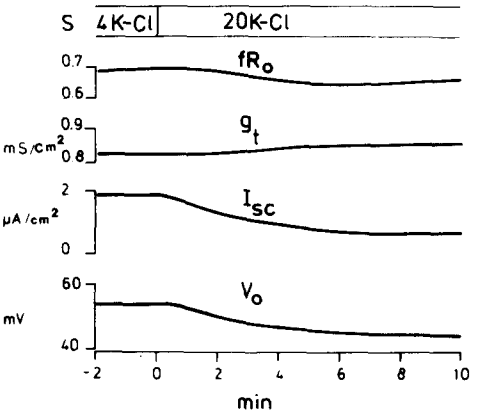

Fig. 1. Effects of increasing stromal K^+ from 4 to 20 mM. Various parameters are plotted versus time. Zero time: when the K^+ concn. was changed. FR_o, apical membrane fractional resistance; g_t, transepithelial conductance in mS/cm^2; I_{sc}, short-circuit current in μA/cm^2; V_o, intracellular potential in mV.

Results

Normal PD responses to changes in K^+ concentration in the stromal solution

The stromal solution K^+ concentration was increased from 4 to 20 or 79 mM in the presence of control solutions. Fig. 1 shows a representative set of responses when stromal K^+ was increased from 4 to 20 mM under short-circuit conditions, and the data from six experiments are summarized in Table I.

The average transepithelial conductance (g_t) and the apical membrane fractional resistance (fR_o) did not change significantly. I_{sc} significantly decreased by 1.4 μA/cm^2 at 2 min and by 3.1 at 10 min after increasing K^+ from 4 to 20 mM from a control value of 6.8 μA/cm^2. V_o decreased by 8.2 mV at 2 min and by 21.3 at 10 min.

Table II shows the changes in the electrical parameters when stromal K^+ was increased from 4 to 79 mM. As in the increase to 20 mM K^+, fR_o did not change. On the other hand, there was a progressive increase in g_t, with an increment at 10 min of 0.50 from a control value of 0.62 mS/cm^2. Changes in I_{sc} and V_o were qualitatively similar to those obtained when stromal K^+ was increased from 4 to 20 mM but they were quantitatively greater.

The 10 min change in V_o per 10-fold increase in stromal K^+ concentration was 30.5 mV for the change in K^+ concentration from 4 to 20 mM and 29.6 mV for the 4 to 79 mM change. This change is about half of the expected 58 mV change if the basolateral membrane had as its conductive pathway only the simple K^+ conductance.

Anomalous PD responses to changes in K^+ concentration in the stromal solution

(1) Under short-circuit current conditions. As previously reported for experiments in the gastric mucosa [11] and cornea [8], anomalous responses were obtained after bathing the epithelia in K^+-free solutions for 20–30 min in the case of the stomach and for 5–10 min in the case of the cornea. Fig. 2A presents data from a typical experiment performed under short circuit current conditions. When stromal K^+ was raised from 0 to 4 mM there was a quick increase in V_o, that is, there was a hyperpolarization of the apical membrane undoubtedly due to an increase of the ($Na^+ + K^+$)-ATPase emf (E_P, Fig. 4) located in the basolateral membrane (see Discussion). In contrast when K^+ was increased from 4 to 20 or 79 mM there was a depolarization of V_o probably due to an increase of E_K (Fig. 4). The I_{sc} increased when stromal K^+ was increased from 0 to 4 mM.

TABLE I

EFFECT OF INCREASING STROMAL K^+ FROM 4 TO 20 mM

Values are means ± S.E. N, number of experiments. Control values obtained before the change in K^+ concn. The other values are the changes obtained, respectively, 2, 5 and 10 min after the change in K^+ concn. Units are: I_{sc}, $\mu A/cm^2$; g_t, mS/cm^2; fR_o, unitless; V_t and V_o, mV. a, $P < 0.01$; b, $P < 0.05$; n.s., $P > 0.05$.

	Control ($N = 6$)	Change in parameter		
		2 min ($N = 6$)	5 min ($N = 6$)	10 min ($N = 5$)
I_{sc}	6.78 ±1.85	−1.35 ±0.41 [b]	−2.24 ±0.68 [b]	−3.06 ±1.09 [b]
g_t	0.576±0.072	0.008±0.01 [n.s.]	0.004±0.014 [n.s.]	0.017±0.012 [n.s.]
fR_o	0.694±0.040	−0.033±0.017 [n.s.]	−0.042±0.023 [n.s.]	−0.018±0.020 [n.s.]
V_o	58.88 ±5.45	−8.22 ±2.67 [b]	−17.88 ±3.83 [a]	−21.26 ±6.79 [b]

TABLE II

EFFECT OF INCREASING STROMAL K^+ FROM 4 TO 79 mM

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

	Control	Change in parameter		
		2 min	5 min	10 min
I_{sc}	5.19 ±0.94 (9)	−4.87 ±0.77 (9) [a]	−8.41 ±1.17 (9) [a]	−10.05 ±1.47 (8) [a]
g_t	0.623±0.106 (9)	0.123±0.022 (9) [a]	0.259±0.041 (9) [a]	0.497±0.104 (8) [a]
fR_o	0.684±0.052 (8)	−0.027±0.018 (8) [n.s.]	−0.006±0.028 (8) [n.s.]	0.027±0.038 (7) [n.s.]
V_o	64.07 ±3.72 (7)	−23.10 ±4.42 (7) [a]	−34.61 ±3.18 (7) [a]	−38.33 ±2.97 (6) [a]

TABLE III

EFFECT OF CHANGING STROMAL K^+ FROM 0 TO 4 mM

Values are means ± S.E. ($N = 21$ experiments). Control values obtained before the change in K^+ concn. The other values are parameter changes at, respectively, 2, 5 and 10 min after the change in K^+ concn., and at the maximal change in V_o (2–5 min). Units as in Table I. a, $P < 0.01$; b, $P < 0.05$; n.s., $P > 0.05$.

	Control	Change in parameter			
		2 min	5 min	10 min	at max. V_o
I_{sc}	5.2 ±0.8	0.9 ±0.1 [a]	1.5 ±0.2 [a]	1.5 ±0.2 [a]	1.0 ±0.2 [a]
g_t	0.545±0.035	−0.009±0.003 [a]	0.007±0.004 [n.s.]	0.029±0.005 [a]	−0.005±0.005 [n.s.]
fR_o	0.672±0.030	0.037±0.008 [a]	0.001±0.012 [n.s.]	−0.042±0.018 [b]	0.036±0.011 [a]
V_o	65.6 ±3.7	7.3 ±1.4 [a]	2.3 ±1.7 [n.s.]	−4.6 ±2.2 [n.s.]	10.1 ±1.4 [a]

TABLE IV

EFFECT OF INCREASING STROMAL K^+ FROM 0 TO 4 mM UNDER OPEN-CIRCUIT CONDITIONS

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

	Control	Change in parameter			
		2 min	5 min	10 min	at max. V_o
g_t	0.592±0.061 (6)	0.097±0.003 (6) [b]	0 ±0.004 (6) [n.s.]	0.008±0.005 (6) [n.s.]	0.007±0.003 (5) [b]
fR_o	0.708±0.048 (6)	0.014±0.009 (6) [n.s.]	−0.039±0.019 (6) [n.s.]	−0.098±0.021 (6) [a]	0.029±0.011 (5) [n.s.]
V_t	8.65 ±2.21 (6)	1.76 ±0.90 (5) [n.s.]	2.80 ±0.88 (5) [b]	2.28 ±0.37 (5) [a]	1.48 ±0.78 (5) [n.s.]
V_o	60.52 ±3.98 (6)	4.32 ±1.86 (6) [n.s.]	−2.13 ±2.26 (6) [n.s.]	−9.77 ±2.95 (6) [b]	7.18 ±1.43 (5) [a]
V_i	69.47 ±3.69 (6)	5.77 ±2.42 (6) [n.s.]	0.18 ±2.60 (6) [n.s.]	−7.92 ±2.75 (6) [b]	8.66 ±1.97 (5) [a]

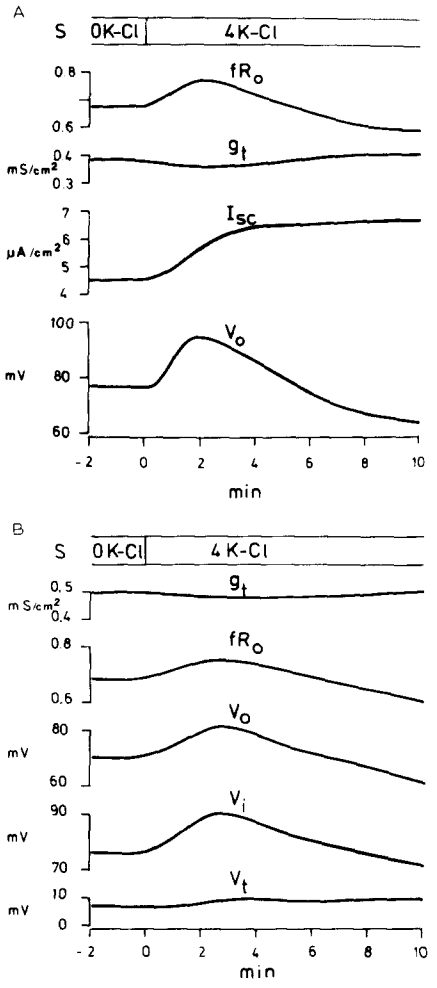

Fig. 2. Effect of increasing stromal K^+ from 0 to 4 mM: (A) under short-circuit conditions; (B) under open-circuit conditions. Cornea exposed to a K^+ free solution for more than 10 min on stromal side before the change in concentration. fR_o and g_t and time as in Fig. 1. V_t, measured transepithelial potential; V_o, transapical membrane potential; V_i, transbasolateral membrane potential ($V_i = V_t + V_o$).

Table III presents data from 21 experiments performed under short-circuit current conditions like the experiment presented in Fig. 2A. Values were from measurements obtained right before stromal K^+ was increased from 0 to 4 mM (control) and at 2, 5 and 10 min after the change in concentration as well as when V_o reached a maximum increase. The maximum V_o increase occurred between 2 and 5 min following the increase in stromal K^+ from 0 to 4 mM. The value of I_{sc} increased progressively reaching an increment of 1.5 $\mu A/cm^2$ at 5 min and remained there at 10 min, from a control value of 5.2 $\mu A/cm^2$. The value of g_t did not change to any great extent but the small decrease of 0.009 mS/cm^2 at 2 min as well as the increase of 0.029 mS/cm^2 at 120 min from a control value of 0.545 mS/cm^2 were statistically significant. The value of fR_o also had very small changes with a significant increase of 0.037 in 2 min and a decrease of 0.042 at 10 min from a control value 0.672. V_o increased initially reaching a maximum of 10.1 mV change between 2 and 5 min, from the control value of 65.6 mV. By 5 and 10 min V_o had returned to the control value.

(2) Under open-circuit conditions. Fig. 2B shows data from a typical experiment performed under open-circuit conditions ($I_{sc} = 0$). The PD across the basolateral membrane, V_i, was obtained from the values of the PD across the apical membrane, V_o, and the transepithelial PD, V_t ($V_i = V_t + V_o$). There was an initial and temporary hyperpolarization of both V_i and V_o and an increase in V_t. Small changes in g_t and fR_o were observed.

Table IV presents data from six experiments performed like the experiment presented in Fig. 2B. A maximum hyperpolarization of V_i and V_o was observed between 2 and 5 min after stromal K^+ had been increased from 0 to 4 mM. This maximum increase was 8.7 mV for V_i and 7.2 mV for V_o over the respective control values of 69.5

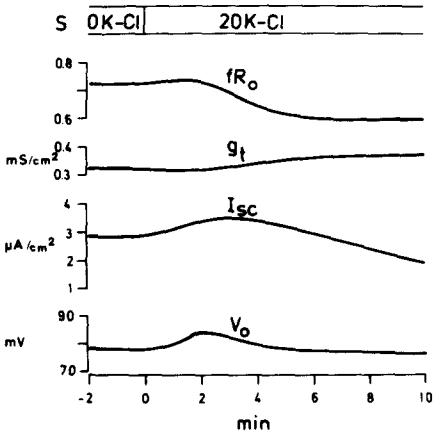

Fig. 3. Effect of increasing stromal K^+ from 0 to 20 mM, after exposure to 0 mM K^+ for more than 10 min. Meaning of time and symbols as in Fig. 1.

TABLE V

EFFECT OF INCREASING STROMAL K^+ FROM 0 TO 20 mM

See legend to Table III. *N*, number of experiments.

	Control ($N = 7$)	Change in parameter			
		2 min ($N = 7$)	5 min ($N = 7$)	10 min ($N = 6$)	At max. V_o ($N = 7$)
I_{sc}	4.56 ±1.18	0.26 ±0.31 n.s.	−0.21 ±0.58 n.s.	−2.36 ±0.75 b	0.11 ±0.10 n.s.
g_t	0.524±0.078	−0.012±0.009 ns.	0.049±0.010 a	0.070±0.020 b	−0.002±0.001 n.s.
fR_o	0.656±0.051	−0.020±0.014 n.s.	−0.073±0.016 a	−0.080±0.027 b	0.020±0.009 n.s.
V_o	48.43 ±4.23	−0.43 ±2.95 n.s.	−11.43 ±2.72 a	−17.12 ±3.45 a	4.56 ±1.10 a

and 60.5 mV. Both V_i and V_o depolarized, respectively, by 7.9 and 9.8 mV below the control level 10 min after the increase in stromal K^+. The increase in V_i is probably due to the activation of the electrogenic ($Na^+ + K^+$)-ATPase pump represented by E_P in Fig. 4. The increase in V_o indicates the presence of an *IR* drop across the apical membrane induced by the activation of the ($Na^+ + K^+$)-ATPase pump with the circuit completed

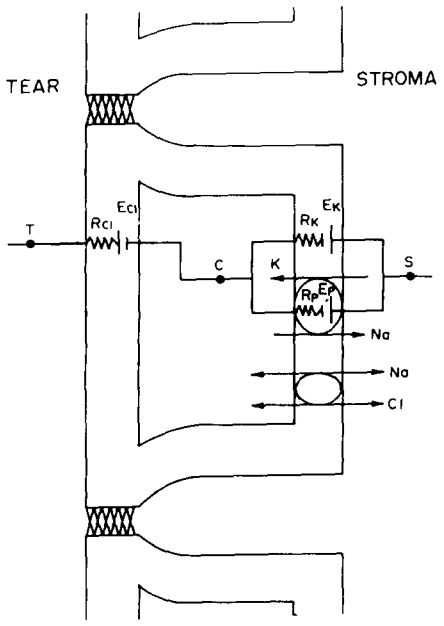

Fig. 4. Schematic representation of some well-accepted pathways in the cornea epithelium. *R* and *E* values are the respective resistances and emf values of the Cl^- conductive pathway in the apical membrane and the simple K^+ conductive and electrogenic ($Na^+ + K^+$)-ATPase pump in the basolateral membrane. Also shown is a Na^+/Cl^- symport in the basolateral membrane.

across the intercellular space (see Discussion). Changes in g_t and fR_o were minimal in open circuit experiments as they were in short circuit current experiments. A small but significant increase in g_t occurred at 2 min and at the max V_o (2–5 min), with no significant changes at 5 or 10 min. A small but significant decrease in fR_o occurred at 10 min with no significant change in this parameter at 2 and 5 min and at the maximum V_o.

Effects of increasing stromal K^+ from 0 to 20 and 79 mM

The response to an increase in stromal K^+ from 0 to 20 mM is presented in Fig. 3. With an increase to 20 mM there were small short-lasting increases in V_o and I_{sc}. There was an increase in g_t and an unexpected decrease in fR_o. The responses of V_o and I_{sc} were a mixture of initial anomalous followed by normal responses.

Table V presents data from seven experiments performed as that presented in Fig. 3. There was an initial hyperpolarization of V_o of 4.6 mV which was followed by a return to the control level at 2 min, and a significant depolarization of 11.4 and 17.1 mV below the control level at 5 and 10 min, respectively, following the increase in K^+ concentration. I_{sc} decreased by 2.4 $\mu A/cm^2$ at 10 min from a control value of 4.6 $\mu A/cm^2$, with no significant changes at 2 min, 5 min or at maximum V_o and g_t did not change initially but decreased by 0.05 and 0.07 ms/cm^2 at 5 and at 10 min, respectively, from a control value of 0.52 mS/cm^2. Changes in fR_o were also observed only at 5 and 10 min with a decrease of 0.07 and 0.08, respectively, from a control value of 0.66.

When stromal K^+ was increased from 0 to 79 mM, changes in all parameters were similar to

those obtained when stromal K^+ was increased from 4 to 79 mM. It appears that the marked increase in stromal K^+ quickly reduced R_K (see Discussion).

Discussion

We have observed two types of responses to changes in the K^+ concentration in the solution bathing the basolateral membrane side of the frog gastric mucosa and frog cornea epithelia [11,14]. Under control conditions changes in the K^+ concentration results in normal PD responses, whereas after bathing in zero K^+ an anomalous response opposite to the normal response results. There is evidence for a simple K^+ conductance pathway in the stromal membrane of the corneal epithelium [5,8] similar to the one in the basolateral membrane of the frog gastric epithelium [14], and the normal PD responses have been explained on the basis of this simple conductance. The anomalous PD responses have been explained on the basis of an electrogenic $(Na^+ + K^+)$-ATPase pump on the basolateral membrane of the gastric mucosa epithelium [11] or of the corneal epithelium [8], that is, the pump transports more Na^+ than K^+ ions per cycle. The presence of the pump in the cornea has been documented by Candia and co-workers [3,15] who have also reported data consistent with transport of more Na^+ than K^+ per cycle [3].

Our conclusions were based on data obtained from transepithelial measurements [8]. It is from data using microelectrodes in presently reported experiments that we can establish the normal as well as anomalous PD responses as due primarily to the direct changes in the PD across the basolateral membrane.

Fig. 4 shows schematically the electrogenic pathways, the simple K^+ conductive $(R_K - E_K)$ pathway and the $(Na^+ + K^+)$-ATPase pump $(R_P - E_P)$ in the basolateral membrane, and the Cl^- conductive $(R_{Cl} - E_{Cl})$ pathway in the apical membrane. From Fig. 4 it follows that

$$V_o = E_{Cl} + I_c R_{Cl} \tag{1}$$

$$V_i = R_K/(R_K + R_P)E_P + R_P/(R_K + R_P)E_K - R_P R_K/(R_K + R_P)I_c \tag{2}$$

where V_o and V_i are the potentials of the tear and stromal solutions, respectively, with reference to the cell, i.e., when V_o and V_i are positive the tear and stromal solutions are positive to the cell. E and R are the respective emf and resistances of the simple Cl^- conductive (Cl), the simple K^+ conductive (K) and the $(Na^+ + K^+)$-ATPase pump (P) pathways. I_c is the transcellular current, considered positive in the direction from tear to stroma; I_c is equal to the sum of the applied current and the current through the intercellular spaces. Under short-circuit conditions the transintercellular current is zero and under open-circuit conditions the magnitude of the transintercellular current equals I_c.

From the definition of apical membrane fractional resistance,

$$fR_o = R_{Cl}[R_{Cl} + R_K R_P/(R_K + R_P)]^{-1} \tag{3}$$

under short-circuit conditions we obtain from Eqns. 1–3

$$V_o = V_i = (1 - fR_o)E_{Cl} + fR_o[E_P R_K/(R_K + R_P) + E_K R_P/(R_K + R_P)] \tag{4}$$

If we assume that the R values do not change, as it usually happened in present experiments, the values of V_o will depend on the emf values of the circuit. When stromal K^+ is increased, E_P should increase and E_K should decrease (see Refs. 8 and 11 for details). A decrease in stromal K^+ should result in the reverse changes of E_P and E_K. The change in V_o will depend on which of the terms containing E_P or E_K dominates. If the term containing E_P dominates, V_o will increase (hyperpolarize) when stromal K^+ is increased and vice versa. If the term containing E_K dominates, the term V_o will decrease (depolarize) when stromal K^+ is increased and vice versa. Changes of V_o presented in this paper suggest that under control conditions (4 mM stromal K) the term containing E_K predominated, that is, V_o decreased (normal response) when stromal K^+ was increased from 4 to 20 or 79 mM. On the other hand, the initial increase in V_o (anomalous response) following the increase in stromal K^+ from 0 to 4 mM after exposure to 0 mM K^+ suggests that the term containing E_P predominates under these conditions.

The prevalence of one or the other terms depends on the magnitude of the coefficients of E_P and E_K at the time stromal K^+ is changed. Thus, under control conditions the resistance of the simple K^+ conductive pathway is relatively low; therefore, the term containing E_K prevails. After exposure to 0 mM K^+, R_K apparently increases to the point where the term containing E_P becomes the dominant one. We assume that R_K depends strongly on the intracellular concentration of K^+, that is, during exposure to zero stromal K^+, the intracellular K^+ decreases which produces an increase in R_K. In support of this concept are recent data obtained in our laboratory [16]. We kept the intracellular K^+ elevated during the period of exposure of the stroma to 0 mM K^+ by adding 10^{-5} M amphotericin B to the tear solution to increase the permeability of the apical membrane to K^+ [3], and increasing the K^+ concentration of the tear solution to 79 mM. Under these conditions we found as expected that the transepithelial PD decreased when stromal K^+ was increased from 0 to 4 mM, i.e., a normal response was obtained indicating the dominance of the term containing E_K and supporting the concept of the importance of the value of R_K in obtaining normal or anomalous responses at the time stromal K^+ is changed. These results indicate that high intracellular K^+ maintains a high K^+ conductance of the simple K^+ pathway even when stromal K^+ equals zero.

Under open-circuit conditions, when stromal K^+ was increased from 0 to 4 mM after exposure to zero K^+, there was an increase in V_i. This increase lasted for a brief period, suggesting the presence of open circuit current from cell to stroma, counter-acting the emf of the pump (see third term of Eqn. 2). The current could be the transport of more Na^+ ions from cell to stroma than K^+ ions from stroma to cell via the pump and/or the movement of K^+ from cell to stroma through the simple K^+ conductance pathway. The circuit may be completed by the movement of Cl^- from cell to tear and, for example, the movement of Na^+ from stroma to tear via the intercellular space. In addition, Na^+ and Cl^- could enter from stroma to cell via the symport. This scheme is supported by data from Klyce [17] who observed in rabbit cornea net movement of Na^+ and Cl^- from stroma to tear under open-circuit conditions.

We note that as the stromal K^+ is increased from 0 to higher and higher concentrations, the anomalous response is diminished and eventually abolished. For example, an increase to 20 mM gives a very small and short-lived anomalous response, while an increase to 79 mM abolishes the response. We surmise that with greater changes in concentration the decrease in the resistance of the simple K^+ conductance pathway is more rapid.

We further note that in the presence of 10^{-3} M ouabain an increase in stromal K^+ from 0 to 4 mM gave no hyperpolarization and no increase in I_{sc}.

In conclusion, data with microelectrodes have been obtained which support the presence of two K^+ conductive pathways in the basolateral membrane of the corneal epithelial cells: a simple conductive pathway and an electrogenic ($Na^+ + K^+$)-ATPase pump transporting more Na^+ than K^+ ions per cycle.

Acknowledgements

This work was supported by grants from NIH (No. EY04759, G.C.) and from Deutsche Forschungsgemeinschaft (W.N). The authors thank Inge Kirmeyer and W. Endstrasser for their excellent technical assistance.

References

1 Zadunaisky, J.A. (1966) Am. J. Physiol. 211, 506–512
2 Candia, O.A. and Askew, W.A. (1968) Biochim. Biophys. Acta 163, 262–265
3 Candia, O.A. and Schoen, H.F. (1978) Am. J. Physiol. 234, F297–F301
4 Candia, O.A. (1982) Chloride Transport in Biological Membranes (Zadunaisky, J.A., ed.), pp. 223–242, Academic Press, New York
5 Reuss, L., Reinach, P.S., Weinman, S.A. and Grady, T.P. (1983) Am. J. Physiol. 244, C336–C347
6 Zadunaisky, J.A., Spring, K.R. and Shindo, T. (1979) Fed. Proc. (Abstr.) 38, 1059
7 Candia, O.A. and Podos, S.M. (1981) Invest. Ophthalmol. Vis. Sci. 20, 733–737
8 Carrasquer, G., Ahn, S., Schwartz, M. and Rehm, W.S. (1985) Am. J. Physiol. 249, F185–F191
9 Carrasquer, G., Rehm, W.S. and Schwartz, M. (1986) Biochim. Biophys. Acta 862, 178–184
10 Carrasquer, G., Rehm, W.S. and Schwartz, M. (1985) Biochim. Biophys. Acta 819, 23–28

11 Schwartz, M., Chu, T.C., Carrasquer, G. and Rehm, W.S. (1981) Biochim. Biophys. Acta 649, 253–261
12 Nagel, W. (1976) Pflugers. Arch. 365, 135–143
13 Nagel, W. and Reinach, P.S. (1980) J. Membrane Biol. 56, 73–79
14 Spangler, S.G. and Rehm, W.S. (1968) Biophys. J. 8, 1211–1227
15 Candia, O.A. (1972) Am. J. Physiol. 223, 1053–1057
16 Carrasquer, G., Luo, R., Rehm, W.S., Schwartz, M. and Dinno, M. (1986) The Physiologist (Abstr.) 29, 121
17 Klyce, S.D. (1975) Am. J. Physiol. 228, 1446–1452