J. Membrane Biol. 56, 73–79 (1980)

The Journal of
Membrane Biology

Mechanism of Stimulation by Epinephrine of Active Transepithelial Cl Transport in Isolated Frog Cornea

Wolfram Nagel and Peter Reinach*

Physiologisches Institut, University of Munich, Munich, West Germany, and Mount Sinai School of Medicine, Departments of Ophthalmology and Physiology, New York, New York 10029

Summary. In the isolated frog cornea, the effects of 0.1 mM epinephrine were measured on both the transepithelial and intracellular electrical parameters. Epinephrine increased the short-circuit current (I_{sc}) and transepithelial electrical conductance (g_t) by 176 and 96%, respectively. The effective electromotive driving force for active transepithelial Cl transport (E_{Cl}) was 45 mV and agrees with the value for E_{Cl} calculated by a different technique in the isolated rabbit corneal epithelium (Klyce, S.D., Wong, R.K.S., 1977, *J. Physiol. (London)* **266**:777). With respect to the tear-side bathing solution, epinephrine caused the intracellular potential difference of short-circuited frog corneas to decrease from -54 to -50 mV ($P > 0.05$). The fractional resistance of the apical membrane $\{F(R_o) = (R_o/R_o + R_i)\}$ where R_o and R_i represent the resistances of the apical and basolateral membranes, respectively, decreased from 0.38 ± 0.06 to 0.23 ± 0.03. Using these values of $F(R_o)$ and the cellular conductances, the calculated Cl resistances of R_o and R_i decreased 4.3- and 2.3-fold, respectively. However, the value for E_{Cl} calculated from the intracellular electrical measurements (48 mV) did not appear to change since this value was in close agreement with the value for E_{Cl} calculated from the effects of epinephrine on the transepithelial electrical parameters. Thus, the effects of epinephrine on I_{sc} and g_t can be accounted for by increases in the Cl conductance of both the apical and basolateral membranes. Epinephrine caused the potential difference across the basolateral membrane to hyperpolarize by 9 mV. All of these results are consistent with the notion that the steps in transepithelial Cl transport include uphill movement into the cell across the basolateral membrane followed by downhill movement across the apical membrane into the tear-side bathing solution.

In the isolated bullfrog cornea, active transepithelial Cl transport from the endothelial to the tear-side bathing solution accounts for about 95% of the short-circuit current (I_{sc}) (Zadunaisky, 1966). This Cl-originated I_{sc} is selectively stimulated by epinephrine (Chalfie, Neufeld & Zadunaisky, 1972). In the isolated rabbit corneal epithelium, results of intracellular electrical measurements of the effects of pulses of 5×10^{-10} M epinephrine in the tear-side bathing solution indicated that active Cl transport is reversibly stimulated by only increasing the apical membrane Cl permeability (Klyce & Wong, 1977). Klyce and Wong suggested that the steps in active Cl translocation towards the tears include uphill movement into the epithelial cell followed by downhill movement out of the cell into the tears. Cl accumulation by this tissue is thought to be a consequence of carrier mediated Na:Cl coupled uptake across the basolateral membrane. The operation of this carrier is dependent on the maintenance of an electrochemical gradient which permits downhill movement of Na across the basolateral membrane into the cell. The maintenance of this gradient is a consequence of basolateral situated (Na:K) ATPase activity. Thus, active Cl translocation across the rabbit cornea is thought to be coupled to the Na gradient across the basolateral membrane.

In the isolated bullfrog cornea, 5×10^{-10} M epinephrine in either the tear or the stromal bathing solution has no effect on the Cl-originated I_{sc}: 10^{-4} M epinephrine in the stroma bathing solution is required to obtain a sustained stimulation of the I_{sc} that lasts long enough to measure reliably changes in the transepithelial Cl fluxes (Montoreano, Candia

* *Mailing address and to whom reprint requests should be made:* Mount Sinai School of Medicine, Ophthalmology Department, One Gustave L. Levy Place, New York, New York 10029.

0022-2631/80/0056-0073 $01.40

& Cook, 1976). Using the higher concentration of epinephrine in the isolated bullfrog cornea, we report here on the effects of this drug on the intracellular electrical parameters of the isolated and short-circuited bullfrog cornea. These measurements were done with microelectrodes, using techniques previously described for frog skin (Nagel, 1976, 1978, and 1979). We found that 10^{-4} M epinephrine increases the Cl conductances of both the apical and basolateral membranes of the epithelial cells and thereby stimulates the Cl-originated I_{sc} without affecting the effective electromotive driving force for Cl transport, E_{Cl}.

Materials and Methods

Bullfrog corneas (*Rana catesbeiana*) were mounted epithelial face up in a Lucite chamber similar to the one previously described (Nagel, 1976). The effective surface area was 0.4 cm^2. A copper grid whose radius of curvature was slightly less than that of the cornea *in vivo* supported the tissue. To avoid edge damage effects, silicone high vacuum grease (Marck Ag, Germany No. 7922) was used to seal the edges of the epithelial half chamber. The perfusion flow rate in the epithelial half chamber (Volume = 0.2 ml) was about 5 ml/min which allowed complete exchange of the tear-side bathing solution within 5 sec. The endothelial half chamber (total volume = 0.3 ml) was perfused at a rate of about 5 ml/min by application of a slightly negative hydrostatic pressure which also helped to secure the cornea to the copper grid. Both sides of the cornea were continuously perfused with NaCl Ringer of the following composition (mM): Na^+, 112; K^+, 2.5; Cl^-, 112; HCO_3^-, 2.5; Ca^{++}, 1; glucose, 5; HPO_4^-, 2. The pH was 8.3 after saturation with air.

List of Symbols

V_t = transepithelial potential difference
I_{sc} = short-circuit current
I_t = transepithelial current flow at $V_t = 0$
V_{sc} = intracellular potential difference under short-circuit conditions ($V_t = 0$)
V_i = potential difference across the basolateral membrane under open-circuit conditions as calculated from Eq. (6) (*see* page 75).
$F(R_o)$ = fractional electrical resistance of the apical membrane – $R_o/(R_o + R_i)$; R_i is the resistance of the basolateral membrane.
g_t = transepithelial electrical conductance
g_c = cellular conductance of active transport pathway
g_s = shunt conductance
g_o, g_i = conductances of the apical and basolateral membranes, respectively (equal to $1/R_o$ and $1/R_i$)
E_o, E_i = equivalent emf's of the apical and basolateral membranes, respectively, when $I_t = 0$.
E_{Cl} = effective electromotive force for transepithelial active Cl transport = $E_i - E_o$

Intracellular recordings were done with microelectrodes prepared from Omega-dot microfiber capillaries of 1.5 mm outer diameter and 0.8 mm inner diameter (Frederick Haer and Co., Ann Arbor, Mich.) on an air-stream cooled microelectrode puller (Brown-Flaming Model P-77, Sutter Instrument Co., San Francisco, Calif.). The microelectrodes were backfilled with 3 M KCl, and their input resistance and tip potential were between 15 and 40 MΩ and less than 5 mV, respectively. Impalement was done from the tear side perpendicular to the surface. A micromanipulator coupled to an ultra-fast stepping motor drive (Fa. Marcinowski, Heidelberg, Germany) permitted the electrode to be advanced in steps as small as 0.125 μm. The electrode position was registered on a meter relative to its first contact with the epithelial surface. The microelectrode potentials were monitored via Ag/AgCl electrodes with a Burr Brown 3621 Instrumentation amplifier (input resistance $>10^{10}\Omega$) with reference to the tear-side solution. The corneas were continuously short-circuited with an automatic voltage clamping device (Helman & Miller, 1971). The transepithelial potential difference was measured with Ag/AgCl electrodes via Ringer-filled bridges which ended 0.5 mm away from the cornea. Transepithelial current was sent through AgCl-coated Ag wire loops which were 4 mm away from the corneal surfaces. Short-circuiting was periodically interrupted every 2–10 sec by clamping the transepithelial potential difference to values of +20 mV for periods of 200 msec. This procedure provided a periodic measurement of the transepithelial electrical conductance in the hyperpolarizing direction ($g_t = \Delta I_t/\Delta V_t$) through the use of two sample hold-amplifiers (Intersil 5110) and appropriate logic circuitry for triggering. A similar circuit was used for continuous recording of the fractional resistance, $F(R_o)$, of the apical membrane relative to the total transcellular resistance: $F(R_o) = R_o/(R_o + R_i) = \Delta V_{sc}/\Delta V_t$. The values of g_t and $F(R_o)$ were recorded along with the I_{sc}, the V_t and the intracellular potential (V_{sc}) on five channels of a multichannel strip chart recorder (BBC-Metrawatt, Nürnberg, West Germany).

Parameters of Equivalent Circuit Model

In Fig. 1, is shown a Thevenin equivalent for each membrane or element of the corneal epithelium. The cellular pathway consists of two "in series" emf's (E_o and E_i) and electrical resistances (R_o and R_i), representing the apical and basolateral membranes, respectively. The cellular pathway is parallel to a passive paracellular pathway represented by R_s. It is assumed that the emf of the latter pathway may be neglected since bathing solutions of identical composition bathe both sides of the cornea. Furthermore, the potential differences and electrical resistances across the endothelium and stroma are not considered since both of these values are neglibibly small compared to those of the epithelium (Klyce & Wong, 1977).

Two methods were used for evaluation of the individual components of the equivalent circuit.

Method 1

Previously, it was shown that active transepithelial Na transport can be formulated by the following relationship (Ussing & Zerahn, 1951):

$$I_{sc} = I_{Na} = g_{Na} E_{Na}$$

where g_{Na} and E_{Na} is the transcellular Na conductance and effective electromotive force for Na transport, respectively. A rapid reproducible technique for the measurement of E_{Na} in the toad bladder was developed more recently (Yonath & Civan, 1971). The application of this method requires that Na is the only transcellular transported ion and that neither g_s, nor E_{Na} are altered upon increasing the I_{sc} with vasopressin. We have applied their technique to the frog cornea for the evaluation of E_{Cl}. The validity of this technique for evaluating E_{Cl} also requires that increasing the I_{sc}

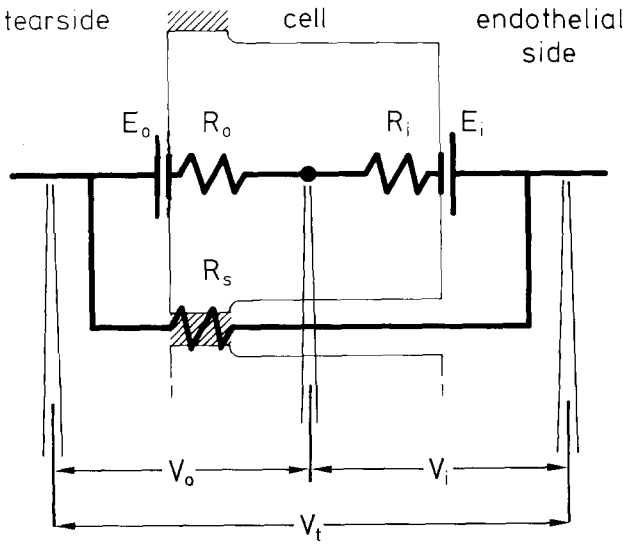

Fig. 1. Thevenin equivalent circuit model of frog corneal epithelium used for determining mechanism of action of epinephrine. R_o and R_i represent the resistances of the apical and basolateral membranes, respectively, between the microelectrode tip and each bathing solution. R_s represents the resistance of the shunt paracellular pathway. E_o and E_i represent the equivalent emf's across the apical and basolateral membranes, respectively. V_o and V_i represent the potential differences between the microelectrode tip and the tear and endothelial bathing solutions, respectively. V_t is the transepithelial potential difference

(i.e., active transepithelial Cl transport) with epinephrine does not alter g_s nor E_{Cl} but only g_c. It has been previously shown that active transepithelial Cl transport accounts for 95% of the I_{sc} (Zadunaisky, 1966).

$$I_{sc}=I_{Cl}=g_c \cdot E_{Cl} \tag{1}$$

Furthermore, the transepithelial electrical conductance, g_t, is equal to the sum of cellular (g_c) and paracellular g_s conductances, i.e.:

$$g_t=g_c+g_s. \tag{2}$$

By combining Eqs. (1) and (2)

$$g_t=I_{Cl}/E_{Cl}+g_s. \tag{3}$$

When corresponding values for g_t and I_{sc} are plotted from experiments in which I_{sc} is increased with epinephrine, the values for g_s and E_{Cl} are obtained from the intercept with the g_t axis at $I_{sc}=0$ and the inverse slope of the regression line, respectively. Values for g_c are calculated for the control and epinephrine periods from the values of g_t and g_s. By use of the simultaneously measured $F(R_o)$, the contributions of the apical and basolateral membrane conductances (g_o and g_i, respectively) can be calculated from

$g_o=g_c/F(R_o)$ and $g_i=g_c\{1-F(R_o)\}$.

Method 2

The potential differences across the individual membranes may be considered to result from emf's and ohmic $R \cdot I$ drops. Across

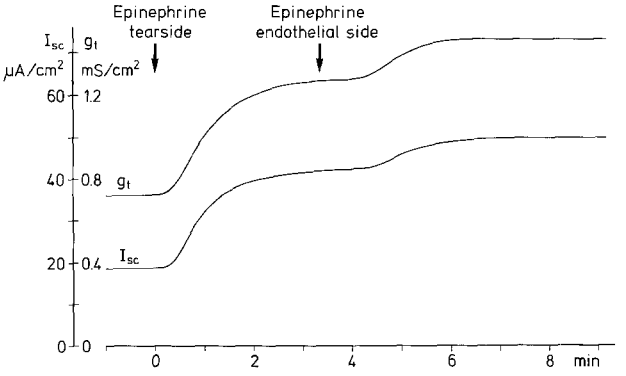

Fig. 2. Effects of epinephrine (0.1 mM) on I_{sc} and g_t

the basolateral membrane under short-circuit conditions, the following relationship applies:

$$V_{sc}=E_i-R_i \cdot I_{sc} \tag{4}$$

substituting $I_{sc}=E_{Cl}/R_{Cl}$ (i.e., Eq. (1)) yields

$$V_{sc}=E_i-\{1-F(R_o)\} \cdot E_{Cl}. \tag{5}$$

Equation 5 is used to estimate E_i, E_{Cl} and E_o ($E_o=E_i-E_{Cl}$). By plotting corresponding values of V_{sc} and $F(R_o)$ from experiments in which $F(R_o)$ is altered. E_i is defined by the intercept of the regression line with the V_{sc} axis where $F(R_o)=1.0$ and E_{Cl} is obtained from the slope. The necessary assumption in using this method is that the changes in $F(R_o)$ can be induced without concomitant alterations of E_o and E_i. Support for the validity of this assumption during the early period after the administration of epinephrine is provided below.

By assuming a linear relationship between V_t and I_t (Klyce, 1971) and the absence of any potential dependent transients, the open-circuit potential difference across the basolateral membrane, V_i, was calculated from measured values of V_{sc}, I_{sc}, g_t, and $F(R_o)$ according to the following relationship:

$$V_i=V_{sc}-I_{sc}/g_t \cdot \{1-F(R_o)\}. \tag{6}$$

All values are means ±SEM. Student t test was used for statistical analysis.

Results

In Figure 2 is shown a representative record of the transepithelial electrical conductance (g_t) and short-circuit current (I_{sc}) before and after the administration of 1×10^{-4} M epinephrine to the tear and then later to the endothelial bathing solution. The addition of epinephrine caused the I_{sc} and g_t to increase essentially with the same time course. To examine the relationship between the increases in g_t and I_{sc} following each of the two additions of epinephrine and to permit use of Method 1, all of the corresponding values of g_t and I_{sc} following each of the two additions of epinephrine from the above experiment were replotted in Fig. 3. Using the criterion of Yonath and Civan for determining whether the I_{sc} increases as a conse-

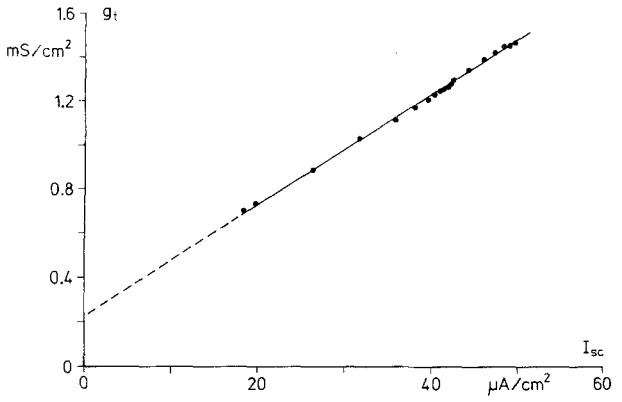

Fig. 3. Plot of g_t *vs.* I_{sc} obtained in the experiment shown in Fig. 2. Values obtained after the addition of epinephrine to tear and endothelial bathing solutions

quence of an increase in g_c, namely, there exists a linear relationship between all of the corresponding values of g_t and I_{sc}, the excellent correlation in Fig. 3 between these parameters suggests that the I_{sc} increased as a consequence of an increase in g_c. Accordingly, g_s, g_c, and E_{Cl} can be evaluated. Linear extrapolation to $I_{sc}=0$ yields a value for g_s of 0.22 mS/cm² and from this values are obtained of 0.45 mS/cm² and 1.23 mS · cm² for the cellular conductance, g_c, before and after the maximal increase in I_{sc} resulting from epinephrine administration, respectively. From the inverse slope of the regression line, a value of 42 mV for E_{Cl} was obtained.

The effects of epinephrine on the transepithelial electrical parameters in nine similar experiments are summarized in Table 1. Epinephrine stimulated the I_{sc} by 176% and increased g_t and g_c by 96 and 184%, respectively. The mean values of g_s and E_{Cl} were 0.24 mS/cm² and 45 mV, respectively, with remarkably little variability between the individual preparations. Values of g_c from the individual experiments are plotted in Fig. 4 as a function of the corresponding I_{sc} values before and after each of the two additions of 10^{-4} M epinephrine. The relationship is highly linear ($r=0.98$) and shows the excellent correlation between I_{sc} and the cellular conductance.

A representative record of V_{sc}, $F(R_o)$ and the basolateral membrane open-circuit potential difference calculated according to Eq. (6) is shown in Fig. 5. The control values of V_{sc} (−55 mV), the basolateral membrane open-circuit potential difference (−70 mV), and $F(R_o)$ (0.60) were stable for more than 3 min before the addition of 10^{-4} M epinephrine to the tear-side solution. Concomitantly with the increase of the I_{sc}, which is not shown, V_{sc} and $F(R_o)$ decreased rapidly to values around −45 mV and 0.3 mV, respectively, whereas the basolateral membrane open-

Table 1. Effects of 10^{-4} M epinephrine on the transepithelial electrical parameters of isolated bullfrog cornea

	I_{sc} (μA/cm²)	g_t (mS·cm⁻²)	g_s (mS·cm⁻²)	g_c (mS·cm⁻²)	E_{Cl} (mV)
Control	11.2±1.7	0.48±0.04	0.24±0.04	0.25±0.04	45.2 ±2.2
Epinephrine	30.9±3.4	0.94±0.09		0.71±0.09	

Values are means ±SEM of nine experiments.

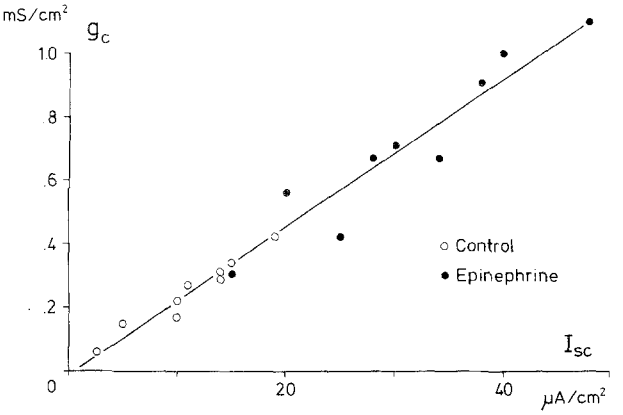

Fig. 4. Values of g_c and I_{sc} during the control period and after maximal increase of these parameters resulting from the addition of 0.1 mM epinephrine to the endothelial bathing solution plotted with respect to one another ($r=0.98$ and $E_{Cl}=44$ mV). Control and epinephrine values are denoted by open and closed circles, respectively ($N=9$)

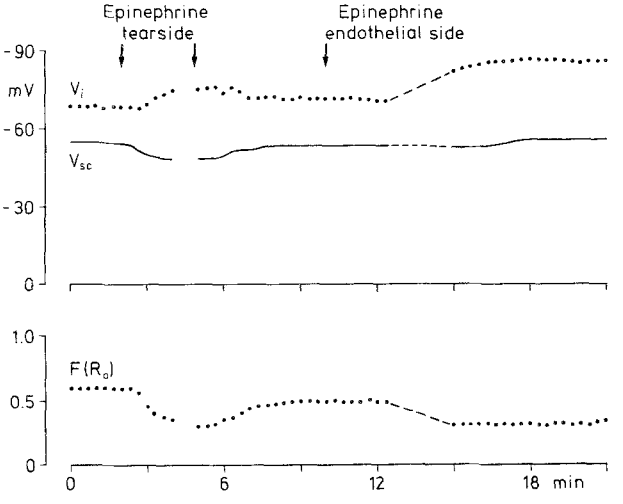

Fig. 5. Time course of the effects of epinephrine (0.1 mM) on open-circuit potential across basolateral membrane, V_i, intracellular potential difference ($V_t=0$), V_{sc} and fractional resistance of apical membrane $\{F(R_o)\}$. From left to right the arrows designate epinephrine addition to tear side, washout from tear side, and addition to endothelial side, respectively. Segment lines represent period of improper impalement

Table 2. Effects of 10^{-4} M epinephrine on intracellular potential and cell membrane resistances of isolated bullfrog cornea

	V_{sc} (mV)	V_i (mV)	$F(R_o)$	R_o (kΩ·cm²)	R_i (kΩ·cm²)
Control	−54±4	−69±5	0.38±0.06	1.6 ±0.4	2.5 ±0.3
Epinephrine	−50±3	−77±4	0.23±0.03	0.37±0.08	1.08±0.10

Values are means ±SEM of nine experiments.

circuit potential difference increased by 7 mV. These changes were partially reversed by removal of epinephrine from the tear-side solution. Subsequent addition of 10^{-4} M epinephrine to the endothelial bathing solution resulted in another decrease of $F(R_o)$ to 0.25 and an increase in the basolateral membrane open-circuit potential difference by 15 mV. The V_{sc}, however, remained unchanged or increased slightly during this period. In all experiments, epinephrine, besides decreasing $F(R_o)$, hyperpolarized the basolateral membrane open-circuit potential difference; the mean increase of the basolateral membrane open-circuit potential difference was 8.6±1.5 mV (Table 2). The V_{sc}, on the other hand, was affected by epinephrine in a rather variable way. The V_{sc} decreased slightly by 4.4±2.4 mV ($P>0.05$) (Table 2). In Table 2 are also shown mean values for $F(R_o)$, R_o, and R_i before and after 10^{-4} M epinephrine administration. R_o and R_i were calculated from g_c with the use of Method I and applying the values of $F(R_o)$. After epinephrine administration, $F(R_o)$ decreased by 40%. Thus, the decrease of the resistance of the apical membrane was more pronounced (77%) than that of the basolateral membrane. Nevertheless, the basolateral membrane resistance decreased significantly by 57%.

In view of the relationship between the changes in g_c and I_{sc}, resulting from the administration of epinephrine, it was of interest to determine whether a similar relationship was observable when comparing changes in g_o and g_i with the epinephrine induced increase in I_{sc}. Figures 6 and 7 show that this was indeed the case. The calculated linear regression lines indicate an excellent correlation between the stimulatory effects of epinephrine on the I_{sc} and the increases in g_o and g_i ($r=0.87$ and 0.96, respectively). In a typical experiment, Fig. 8 shows the responses of V_{sc} and $F(R_o)$ to the addition of 10^{-4} M epinephrine to the tear-side bathing solution. In order to permit application of Method II, the corresponding values of V_{sc} and $F(R_o)$ after the addition of epinephrine to the tear-side bathing solution were replotted in Fig. 9. The corresponding values of V_{sc} and $F(R_o)$ are excellently described by a linear regression line ($r\approx 1.0$).

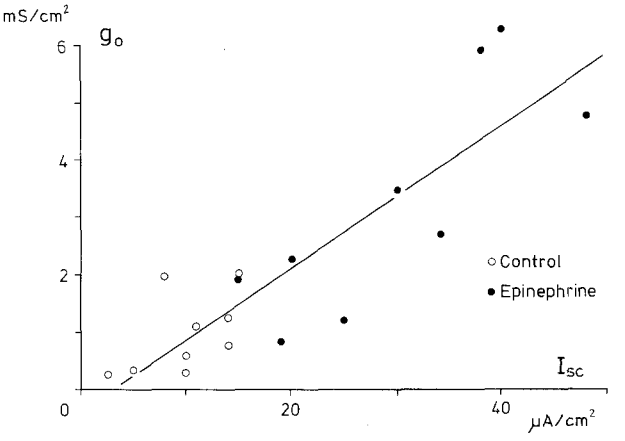

Fig. 6. Values of g_o and I_{sc} during the control period and after maximal increase of these parameters resulting from the addition of 0.1 mM epinephrine to the endothelial bathing solution plotted with respect to one another ($r=0.87$). Control and epinephrine values are denoted by open and closed circles, respectively ($N=9$)

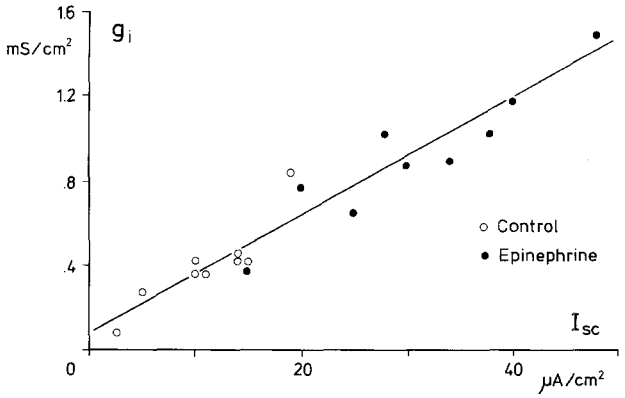

Fig. 7. Values of g_i and I_{sc} during the control period and after maximal increase of these parameters resulting from the addition of 0.1 mM epinephrine to the endothelial bathing solution plotted with respect to one another ($r=0.96$). Control and epinephrine values are denoted by open and closed circles, respectively ($N=9$)

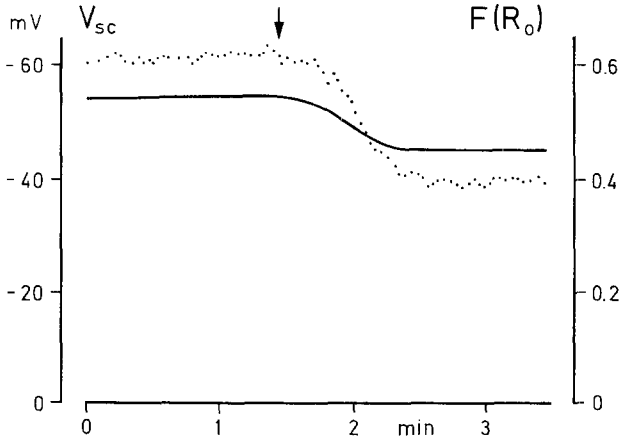

Fig. 8. Time course of the effects of epinephrine (0.1 mM) in the tear-side bathing solution on V_{sc} (. .) and $F(R_o)$ (—)

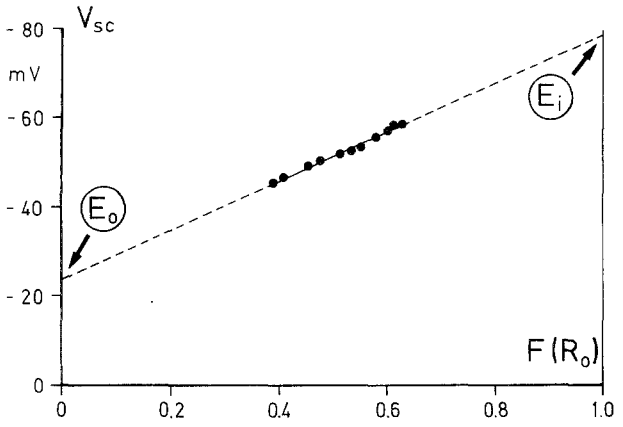

Fig. 9. Correlation between V_{sc} and $F(R_o)$ in a typical experiment during the control period and progressive increase in I_{sc} resulting from the addition of epinephrine (0.1 mM) to the tear-side bathing solution. The intercepts with the Y axes represent E_o {at $F(R_o)=0$} and E_i {at $F(R_o)=1.0$}. The slope of the line is equal to E_{Cl}

By extrapolating to V_{sc} at $F(R_o)=0$ and 1.0, values for E_o and E_i were obtained, respectively. In this particular experiment, E_o, E_i, and E_{Cl} were 24, 79, and 55 mV, respectively. In five experiments, the mean values for E_o, E_i, and E_{Cl} were 38 ± 1 mV, 86 ± 5 mV, and 48 ± 1 mV, respectively. Since in this analysis only the initial changes in V_{sc} and $F(R_o)$ were considered, it is unlikely that there is interference from any secondary effects of epinephrine.

Discussion

Based on the results of studies in the isolated rabbit corneal epithelium (Klyce & Wong, 1977) and the rectal gland of the dogfish (Silva et al., 1977), it appears that active Cl transport is secondary in that it is coupled to the Na gradient maintained across the basolateral membrane by the (Na:K)ATPase pump mechanism. The current notion for the steps in Cl translocation in these tissues include uphill movement of Cl across the basolateral membrane against an electrochemical gradient followed by passive downhill movement out of the cell across the apical membrane. This proposed mechanism also appears tenable in the frog cornea since it has been shown that the intracellular Cl activity averages 29 mM and the intracellular open-circuit potential is -47 mV which indicate that Cl is pumped into the cell against an electrochemical gradient across the basolateral membrane (Zadunaisky, Spring & Shindo, 1979). In order for Cl to be pumped out of the cell across the apical membrane, the Cl activity would have to be approximately threefold less than the measured value.

The results of our measurements of the effects of epinephrine on the transepithelial and intracellular electrical parameters further substantiate the utility of this model in explaining the steps involved in active transepithelial Cl transport across the frog cornea. Furthermore, from our approach it is possible to calculate values for each of the electrical elements in the Thevenin equivalents of the apical and basolateral membranes. The calculation of these values provides information about the relative Cl permeabilities of the two membranes and the effective electromotive force for active transepithelial Cl transport, E_{Cl}.

We have used two different methods to calculate values for the elements of the electrical equivalent circuit shown in Fig. 1. With method I, measurements of the transepithelial electrical parameters (i.e., g_t and I_{sc}) were used to calculate values for g_s, the lumped parameters g_c ($g_c=g_o+g_i$) and $E_{Cl}(E_i-E_o)$. The use of this method seems reasonable for two reasons. First of all, there was an excellent linear correlation between corresponding values of g_t and I_{sc} following the addition of epinephrine to the tear and endothelial side bathing solutions. Secondly, the calculated value for g_s (0.24 mS·cm^{-2}) obtained with Method I is in close agreement with the value calculated from the partial conductance equation and the measurement of unidirectional Na and Cl fluxes ($g_s=$ 0.22 mS·cm^{-2}) (Reinach & Candia, 1978). These two considerations suggest that the effect of epinephrine on I_{sc} stem from an increase in g_c rather than g_s.

In the rabbit corneal epithelium E_{Cl} was measured from the effects of epinephrine on the transepithelial potential difference as a function of prestimulation potential at open circuit (Klyce & Wong, 1977). From a plot of the change in the transepithelial potential difference as a function of prestimulation potential difference at open circuit, E_{Cl} is obtained from the intercept of the line relating these two parameters. The intercept value represents the minimum transepithelial potential difference required to fully inhibit net Cl translocation across the apical membrane and was found to be 45 mV which is in agreement with our value for E_{Cl}.

The measurement of the intracellular electrical parameters {V_{sc} and $F(R_o)$} permitted us to calculate the individual values of g_o and g_i. Furthermore, with the use of Method II, values for E_o and E_i were also calculated .Regardless of whether epinephrine was added to the tear-side or endothelial-side bathing solution $F(R_o)$ always decreased and V_i hyperpolarized. These effects indicate that epinephrine increased both g_o and g_i. The extent of the increases in g_o and g_i appeared to be dependent upon the bathing solution to which epinephrine was added. The addition of epinephrine to the tear-side bathing solution depolarized V_{sc} even though V_i hyperpolarized. These results suggest that with this type of addition the increase in g_c was predominantly due to an increase of g_o

even though g_i also increased. The addition of epinephrine to the endothelial bathing solution caused V_i to hyperpolarize more than with the former addition and the V_{sc} did not change from the control value. Furthermore, $F(R_o)$ further declined to its minimal value. All of these effects indicate that the increase in g_i was larger than when epinephrine was only added to the tear-side bathing solution. These effects of epinephrine on g_o and g_i are different than in the rabbit cornea. In the rabbit cornea, epinephrine addition to the tear-side bathing solution selectively increased the Cl permeability of the apical membrane (Klyce & Wong, 1977). However, it should be mentioned that in the rabbit cornea 5×10^{-10} M epinephrine was effective in mediating this effect on Cl permeability, whereas in the frog cornea 1×10^{-4} M epinephrine had to be used to obtain a sustained change in the electrical parameters.

It is of interest to consider the relative permeability properties of the apical and basolateral membrane to Cl, Na and K. As in the rabbit cornea, our results suggest that the apical membrane is selectively permeable to Cl since the calculated value of the apical membrane equivalent emf ($E_o = 38$) predicts from the Nernst equation an intracellular Cl activity of 25 mM, which is in close agreement with the measured value of 29 mM.

We found under control conditions that R_i is 2.5 $k\Omega cm^2$. In an earlier study with the frog cornea, it was shown that the cellular conductance to Na and K is at the most about 0.04 $mS \cdot cm^{-2}$, and since the apical membrane is primarily Cl permeable it would appear that the cellular Na and K conductance is localized to the basolateral membrane (Reinach & Candia, 1978). Since R_i is equal to 2.5 $k\Omega \cdot cm^2$ and R_{NaK} is approximately 25 $k\Omega cm^2$ (i.e., 1/0.04), the value of R_{Cl} in parallel with R_{NaK} is about 2.8 $k\Omega cm^2$. Therefore, the value for R_i is primarily reflective of Cl permeability since the Cl permeability appears to be ninefold larger than the Na and K permeability.

With Method II, we calculated a value for E_{Cl} based on measurements of the intracellular parameters. We found that E_{Cl} calculated with Methods I and II are supportive of the concept that the increase in I_{sc} mediated by epinephrine is a consequence of an increase only in g_c rather than E_{Cl}. Therefore, epinephrine appears to be an effective probe for evaluating E_{Cl} since E_{Cl} appears to be invariant upon increasing I_{sc}.

An interpretation of what E_i is representative of cannot be made without knowing the intracellular Na and K activities. If it is assumed that the intracellular K activity in the frog cornea is the same as in the rabbit (i.e., 114 mM) and that E_i is representative of a K diffusion potential, then our value of 86 mV for E_i is 10 mV less negative than the predicted value (Hansen et al., 1979).

In summary, we have shown that epinephrine can be used to measure E_{Cl} in the frog cornea since it increases the I_{sc} by increasing the Cl conductances of both the apical and basolateral membranes without affecting E_o or E_i or E_{Cl}. Furthermore, our results are consistent with the current notion of the mechanism of active transepithelial Cl transport in the frog cornea and suggest that Cl uptake occurs against an electrochemical gradient of -35 mV across the basolateral membrane, followed by passive diffusion into the tears along a favorable electrochemical gradient of 12 mV.

We thank L. Reuss for his comments on a preliminary version of this paper.

This work was supported by grants from Deutsche Forschungsgemeinschaft (W. Nagel) and N.E.I. grant # EYO-1976 (P. Reinach).

References

Chalfie, M., Neufeld, A.H., Zadunaisky, J.A. 1972. Action of epinephrine and other cyclic AMP-mediated agents on the chloride transport of the frog cornea. *Invest. Ophthalmol.* **11**:644

Hansen, L.L., Koch, M., Platsch, K.D., Wiederholt, M. 1979. Intracellular potassium activity of isolated human and rabbit corneal epithelium. *Exp. Eye Res.* **29**:367

Helman, S.I., Miller, D.A. 1971. *In vitro* techniques for avoiding edge damage in studies of frog skin. *Science* **173**:146

Klyce, S.D. 1971. Electrophysiology of corneal epithelium. Ph.D. Thesis. Yale University, New Haven

Klyce, S.D., Neufeld, A.H., Zadunaisky, J.S. 1973. The activation of chloride transport by epinephrine and Db cyclic-AMP in the cornea of the rabbit. *Invest. Ophthalmol.* **12**:127

Klyce, S.D., Wong, R.K.S. 1977. Site and mode of adrenalin action on chloride transport across the rabbit corneal epithelium. *Am J. Physiol.* **266**:777

Montoreano, R., Candia, O.A., Cook, P. 1976. α and $-\beta$ adrenergic receptors in regulation of ionic transport in frog cornea. *Am J. Physiol.* **230**:1487

Nagel, W. 1976. The intracellular electrical potential profile of the frog skin epithelium. *Pfluegers Arch.* **365**:135

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

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

Reinach, P.S., Candia, O.A. 1978. Effects of tryptamine on active transepithelial Na and Cl transport in the isolated bullfrog cornea. *Biochim. Biophys. Acta* **510**:327

Silva, P., Stoff, J., Field, M., Fine, L., Forrest, J.N., Epstein, F. 1977. Mechanism of active chloride secretion by shark rectal gland: Role of Na-K-ATPase in chloride transport. *Am. J. Physiol.* **233(4)**:F298

Ussing, H.H., Zerahn, K. 1951. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta. Physiol. Scand.* **23**:110

Yonath, J., Civan, M.M. 1971. Determination of the driving force of the Na^+ pump in toad bladder by means of vasopressin. *J. Membrane Biol.* **5**:366

Zadunaisky, J.A. 1966. Active transport of chloride in frog cornea. *Am. J. Physiol.* **211**:506

Zadunaisky, J.A., Spring, K.R., Shindo, T. 1979. Intracellular chloride activity in the frog corneal epithelium. *Fed. Proc.* **38**:1059