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Effect of Dicyclohexylcarbodiimide (DCCD) on Transport Parameters in the Frog Cornea Epithelium

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Abstract. Dicyclohexylcarbodiimide (DCCD) is a carboxyl group modifier and it is an inhibitor of various ATPases. Present experiments, using an in vitro preparation, were designed to study whether DCCD affected the transporters of the bullfrog cornea epithelium, specifically, the Na^+/K^+ ATPase pump located in the basolateral membrane. For this purpose, corneas were impaled with microelectrodes and experiments were done under short-circuit current (I_{sc}) conditions. Addition of DCCD to a concentration of 10^{-4} M to the tear solution gave a marked decrease in I_{sc}; a marked depolarization of the intracellular potential, V_o; and a significant decrease in the apical membrane fractional resistance, fR_o. There were small and variable although significant changes in the transepithelial conductance, g_t. The effects may be explained by a decrease in the basolateral membrane K^+ conductance, in combination with a partial inhibition of the Na^+/K^+-ATPase pump located in the basolateral membrane. There is also evidence for an increase in the apical membrane Cl^- conductance.

Key words: Dicyclohexylcarbodiimide — Frog cornea (*R. catesbeiana*) — Na^+/K^+-ATPase — K^+ conductance — Short-circuit current — Microelectrode technique

Introduction

Dicyclohexylcarbodiimide (DCCD) has been used as a coupling agent in the peptide synthesis (Sheehan & Hess, 1955; Khorana, 1955). DCCD is a carboxyl group modifier (Hoare & Hoshland, 1967) that has been found to affect the renal Na^+/H^+ exchanger (Friedich, Sablotni & Burkhard, 1986) and the renal cortical Na^+/HCO_3^- cotransporter (Ruiz & Arruda, 1992; Bernardo, Kear & Arruda, 1997). DCCD is an ATPase inhibitor mostly affecting the F_1F_0 H-ATPases (Fillingame, 1976) and the vacuolar H-ATPases (Forgac, 1989). The phosphorylated ATPases are affected to a lesser extent by DCCD (*see* Table 1, Forgac, 1989).

The frog cornea has an epithelium that provides the means for easy and reproducible physiological studies with or without microelectrodes. For this reason, it is an excellent model to test the biological effects of toxic and other substances that affect epithelial transport. Cl^- is actively transported by the cornea epithelium from stroma to tear. The primary active transport is the Na^+/K^+ ATPase pump located in the basolateral membrane (Candia, Bentley & Cook, 1974; Carrasquer et al., 1985; Carrasquer et al., 1987). Other transporters that play an important role on Cl^- secretion are the Cl^- conductance located in the apical membrane (Nagel & Reinach, 1980; Reus et al., 1983; Nagel & Carrasquer, 1989) and the K^+ conductance (Candia, Bentley & Cook, 1974; Carrasquer et al., 1985, 1987) and the NaCl symport located in the basolateral membrane (Zadunaisky, 1972; Frizzel, Field & Schultz, 1979; Candia, 1982; Reus et al., 1983; Nagel & Carrasquer, 1989). Study of effects of toxic or other substances of the corneal epithelium is important. Although the endothelium is responsible for corneal transparency, the epithelium contributes to this function.

In the present study on the frog cornea epithelium, we have found that DCCD has definite effects on the basolateral membrane K^+ conductance, the Na^+/K^+ ATPase pump and the apical membrane Cl^- conductance.

Materials and Methods

Bullfrog corneas (*Rana catesbeiana*) were mounted tear side up in a lucite chamber as previously described (Nagel, 1976; Nagel & Reinach,

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1980). 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 or tear) chamber (0.2 ml) with the lower (stroma) chamber (0.3 ml). Note that stroma chamber or solution is used throughout the paper with reference to chamber or solution closest to the stroma area of the cornea. Both chambers were continuously perfused at a rate of about 5 ml/min to insure complete exchange in 5–10 sec. A slight negative hydrostatic pressure was applied to the lower chamber to help secure the cornea to the copper grid. Control (regular) solutions contained (in mM) (stroma): Na^+, 102; K^+, 4.2; Ca^{2+}, 1; Mg^{2+}, 0.8; Cl^-, 106.2; SO_4^{2-}, 0.8; phosphate 1; and glucose 10; (tear): Na^+, 100; K^+, 4; Ca^{2+}, 1; Cl^-, 97; HCO^{3-}, 5; phosphate 2; and glucose 10. K^+ was substituted for Na^+ in high K^+ stroma solutions. In experiments where tear K^+ was increased in the presence of amphotericin B, the control solutions had (in mM): Na^+, 27 and choline 75, then K^+ was substituted for choline in high K^+ solutions. Na^+ was substituted with choline in low Na^+ concentration solutions. Cl^- was substituted with sulfate in low Cl^- concentration solutions and sucrose was added for correction of the osmolality.

In experiments reported in this paper, DCCD was added to the tear solution to a final concentration of 10^{-4} M (MW 206.3). This concentration was obtained by addition of 0.1 ml of 10^{-1} M DCCD in alcohol to 100 ml of final bathing solution. Pilot experiments in which the concentrations were below 10^{-4} M showed no or minimal effects. DCCD was added to the stroma solution up to a final concentration of 10^{-4} M and 10^{-3} M. When used, amphotericin B was added to the tear solution to a final concentration of 10^{-5} M.

Typical experiments were performed with a pH in the stroma solution of 7.3–7.4 and a pH of the tear solution of 8.5–8.6. Candia (1973) showed that high pH in the tear solution was favorable for high I_{sc} and Cl^- fluxes.

To rule out the lower pH as the reason for smaller effects of DCCD when added to the stroma solution, experiments were performed with the same solutions and pH were used on both sides of the cornea. In these experiments, all solutions had 25 mM/l HCO_3^- and were gassed with 5% CO_2 and 95% O_2. The pH of the solutions was 7.3–7.4.

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_o, was recorded with 3 M KCl-filled microelectrodes which had an input resistance of 50–70 Mohm. V_o was recorded with reference to the tear solution. Corneas were short-circuited using an automatic clamp device (Biomedical Instruments, Germering, FRG) except for brief perturbations that lasted about 200 msec, during which the transepithelial potential was clamped at +10 mV (stroma side positive). These perturbations were repeated every 1–2 sec 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}) in $\mu A/cm^2$; g_t, in mS/cm^2; fR_o, unitless; and V_o, in mV, 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_o is defined as an increase in the negativity of the intracellular potential. Depolarization is regarded as the opposite of hyperpolarization.

Student's *t*-test with paired observations was performed to determine the level of significance when the data could be paired. Otherwise, Student's *t*-test with unpaired observations was used.

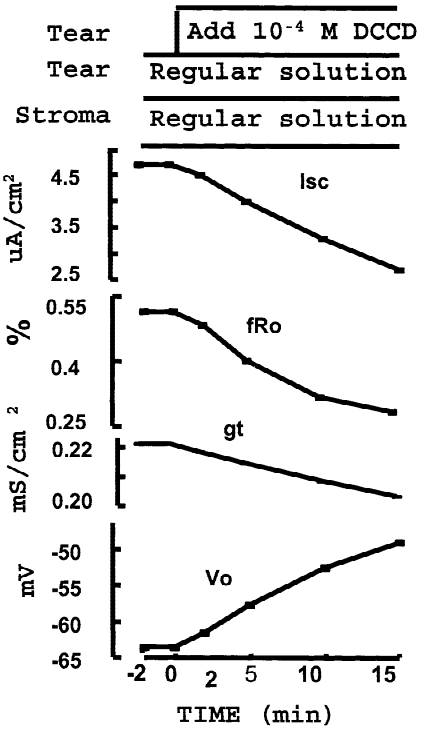

Fig. 1. Effect of 10^{-4} M DCCD in the tear solution. The pH was 7.3 in the bathing solutions. Values are means from 6 experiments. Short-circuit current, I_{sc}, in $\mu A/cm^2$; apical membrane fractional resistance, fR_o, unitless; transepithelial conductance, g_t, in mS/cm^2; intracellular potential, V_o, in mV; all parameters are plotted *vs.* time. Zero time when DCCD was added.

Results

Effect of Adding DCCD to a Concentration of 10^{-4} M in the Cornea Tear Solution

Because of the smaller response to DCCD when it was added to the stroma solution (pH 7.3) (*see below*), experiments were performed at two different pHs in the tear solution, namely, 7.3 and 8.5. The stroma pH in these experiments was maintained at 7.3. Figure 1 shows the effects of DCCD when added to the tear solution with pH of 7.3 in both solutions. The curves present the mean values, from six experiments, of the short-circuit current, I_{sc}, the apical membrane fractional resistance, fR_o, the transepithelial conductance, g_t, and the intracellular potential, V_o, plotted *vs.* time, with zero being the time of addition of DCCD.

While Fig. 1 shows the typical time course of a set of experiments, Table 1 presents numerical data of the mean control values and the mean changes of the parameters at 15 min after addition of DCCD for all experiments. The left two columns of Table 1 present the data obtained at pH 8.5 in the tear solution. I_{sc} decreased by

Table 1. Effects of adding 10^{-4} M DCCD to tear solution with pH 7.3 in stroma solution and with two different pHs of 8.5 and 7.3 in tear solution

	Control Tear pH 8.5	Change in parameter	Control Tear pH 7.3	Change in parameter
	(7 experiments)		(6 experiments)	
I_{SC}	4.4 ± 0.5	−2.7 ± 0.2[a]	4.7 ± 0.6	−2.0 ± 0.1[a]*
fR_o	0.55 ± 0.05	−0.26 ± 0.03[a]	0.52 ± 0.02	−0.23 ± 0.03[a]
g_t	0.28 ± 0.02	0.01 ± 0.004[b]	0.23 ± 0.01	−0.03 ± 0.006[a]*
V_o	−63.3 ± 3.3	25.7 ± 1.9[a]	−63.5 ± 3.6	14.5 ± 1.1[a]*

Values are means ± SE. Control values obtained before the addition of DCCD. The other values are the changes obtained 15 min after the addition of DCCD. Units are: I_{SC}, μA/cm^2; g_t, *mS*/cm^2; fR_o, unitless; V_o, mV.

[a] $P < 0.01$; [b] $P < 0.05$.

* $P < 0.01$ when comparing the effects of DCCD with pH 8.5 *vs.* pH 7.3 in tear solution (Student's *t*-test with *unpaired* observations).

2.7 from 4.4 μA/cm^2 control; fR_o decreased by 0.26 from 0.55 control; g_t had a very small but significant increase of 0.01 from 0.28 mS/cm^2 control; and V_o, depolarized by 25.7 from −63.3 mV control. The right two columns (same experiments as in Fig. 1) show that the effects of DCCD at pH of 7.3 were similar to those at pH 8.5 in the tear solution only for fR_o. The effects on other parameters, that is, on I_{sc}, g_t and V_o, were significantly different at tear pH 7.3 than at tear pH 8.5: I_{sc} decreased less at pH 7.3 than at pH 8.5 (2 *vs.* 2.7 μA/cm^2); g_t decreased by 0.03 mS/cm^2 at pH 7.3 while it significantly increased by 0.01 mS/cm^2 at pH 8.5; and V_o depolarization was smaller at pH 7.3 than at pH 8.5 (14.5 *vs.* 25.7 mV).

Effect of Adding DCCD to a Concentration of 10^{-4} M and 10^{-3} M in the Cornea Stroma Solution

These experiments were done at pH 7.3 in the stroma and pH 8.5 in the tear solution.

The left two columns of Table 2 show data on the effects of DCCD when added to a concentration of 10^{-4} M in the stroma solution (seven experiments). I_{sc} decreased by 0.7 from 4.6 μA/cm^2 control; fR_o decreased by 0.05 from 0.65 control; g_t did not change; and V_o depolarized by 8.9 from −63.3 mV control.

Since the effects of 10^{-4} M DCCD in the stroma solution were markedly smaller than when the same concentration was used in the tear solution, experiments were performed with 10^{-3} M DCCD in the stroma solution. The data from five experiments are presented in the right two columns of Table 2. With 10^{-3} M DCCD in the stroma solution, I_{sc} decreased by 1.6 from 4.9 μA/cm^2 control; fR_o decreased by 0.16 from 0.48 control; g_t decreased 0.02 from 0.28 mS/cm^2 control; and V_o, depolarized by 15.6 from −55.9 mV control.

Table 2. Effects of adding 10^{-4} M and 10^{-3} M DCCD to stroma solution

	Control 10^{-4} M DCCD	Change in parameter	Control 10^{-3} M DCCD	Change in parameter
	(7 experiments)		(5 experiments)	
I_{SC}	4.6 ± 0.6	−0.7 ± 0.1[a]	4.9 ± 0.5	−1.6 ± 0.3[a]*
fR_o	0.65 ± 0.03	−0.05 ± 0.01[a]	0.48 ± 0.05	−0.16 ± 0.02[a]*
g_t	0.25 ± 0.01	0.0 ± 0.0[ns]	0.28 ± 0.01	−0.02 ± 0.01[b]*
V_o	−63.3 ± 2.7	8.9 ± 0.8[a]	−55.9 ± 2.6	15.6 ± 2.0[a]*

Values are means ± SE. Symbols and units as in Table 1.

[ns] $P > 0.05$.

* = $P < 0.02$ when comparing the effects of 10^{-4} M with 10^{-3} M DCCD (Student's *t*-test with *unpaired* observations).

The effects of adding DCCD to the stroma solution were significantly greater with 10^{-3} M were than with 10^{-4} M but still were smaller than with 10^{-4} M in the tear solution.

Because of the significant effect of DCCD on fR_o, with minimal and variable effects on g_t, we decided to study the effect of DCCD on the two main conductance pathways, the K^+ conductance in the basolateral membrane and the Cl^- conductance in the apical membrane. The ion substitution method was used for this purpose.

Effects of 10^{-4} M DCCD in the Tear Solution on the Response of the Transport Parameters to a Change in Stroma K^+ Concentration from 4 to 79 mM

Figure 2 shows the time course of the effect of increasing stroma K^+ concentration in the six experiments studied, before adding DCCD to the tear solution.

Table 3 presents the mean control values and the mean changes of the parameters at 10 min after increasing stroma K^+ from 4 to 79 mM in six experiments. The left two columns of Table 3 present the data obtained before DCCD: I_{sc} decreased by 5.2 from 3.3 μA/cm^2 in 4 mM K^+, that is, it became negative; fR_o did not change; g_t increased by 0.06 from 0.26 mS/cm^2 in 4 mM K^+; and V_o, depolarized by 40.5 from −71.8 mV in 4 mM K^+. The right two columns of Table 3 present the data obtained with DCCD in the tear solution, at least 30 min before and after changing stroma K^+: I_{sc} decreased by 2.3 from 0.5 μA/cm^2 in 4 mM K^+; fR_o did not change; g_t increased slightly, but significantly, by 0.03 from 0.22 mS/cm^2 in 4 mM K^+; and V_o, depolarized by 25.2 from −49.0 mV in 4 mM K^+. The changes in I_{sc} (2.3 *vs.* 5.2 μA/cm^2) and V_o (25.2 *vs.* 40.5) due to the change in stroma K^+ were significantly lower in the presence of DCCD. The effects of increasing stroma K^+ from 4 to 79 mM on fR_o and g_t were not affected by DCCD.

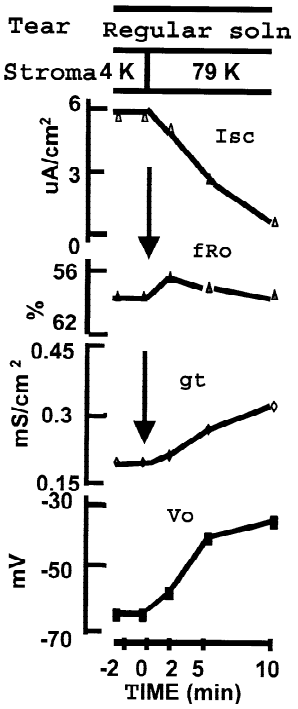

Fig. 2. Effect of changing the concentration of K^+ in the stroma solution from 4 to 79 mM. Values are means from 8 experiments before DCCD. Symbols as in Fig. 1. Zero time when K^+ concentration was changed.

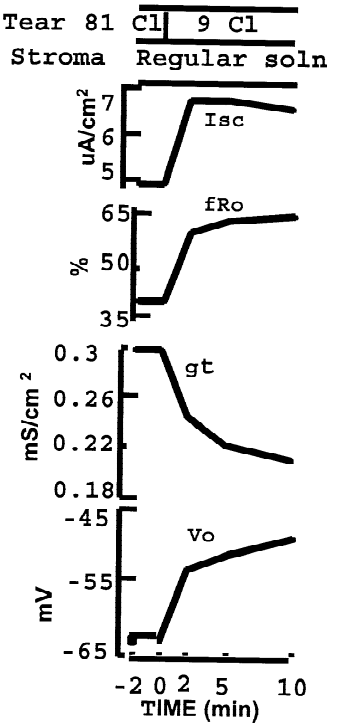

Fig. 3. Effect of changing the concentration of Cl^- in the tear solution from 81 to 9 mM. Values are means from 7 experiments. Symbols as in Fig. 1. Zero time when Cl^- concentration was changed.

Table 3. Effects of changing stroma K^+ concentration from 4 to 79 mM without and with 10^{-4} M DCCD in the tear solution (6 experiments)

	Control	Δ = 10 min	Control	Δ = 10 min
	Without DCCD		With DCCD	
I_{SC}	3.3 ± 0.3	−5.2 ± 0.4[a]	0.5 ± 0.2	−2.3 ± 0.3[a]*
fR_o	0.62 ± 0.05	−0.03 ± 0.03[ns]	0.56 ± 0.03	−0.03 ± 0.02[ns]
g_t	0.26 ± 0.04	0.06 ± 0.02[a]	0.22 ± 0.02	0.03 ± 0.004[a]
V_o	−71.8 ± 2.8	40.5 ± 2.6[a]	−49.0 ± 3.6	25.2 ± 2.2[a]*

Symbols and units as in Table 1. Control values obtained before the change in K^+ concentration. The other values are the changes obtained 10 min after the change in K^+ concentration.
* $P < 0.01$ when comparing the effects of changing stroma K^+ in the presence *vs.* absence of DCCD (Student's *t*-test with *unpaired* observations).

Table 4. Effects of changing tear Cl^- concentration from 81 to 9 mM without and with 10^{-4} M DCCD in the tear solution (7 experiments). pH 7.3 in both solutions.

	Control	Δ = 10 min	Control	Δ = 10 min
	Without DCCD		With DCCD	
I_{SC}	4.6 ± 0.58	2.2 ± 0.4[a]	0.79 ± 0.6	4.4 ± 0.7[a]*
fR_o	0.41 ± 0.03	0.24 ± 0.01[a]	0.33 ± 0.03	0.29 ± 0.01[a]*
g_t	0.29 ± 0.02	−0.1 ± 0.01[a]	0.38 ± 0.03	−0.15 ± 0.02[a]*
V_o	−62.9 ± 1.3	13.8 ± 2.4[a]	−48.6 ± 2.4	12.4 ± 1.6[a]*

Symbols and units as in Table 1. Control values obtained before the change in Cl^- concentration. The other values are the changes obtained 10 min after the change in Cl^- concentration.
* = $P < 0.01$ when comparing the effects of changing tear Cl^- in the presence *vs.* absence of DCCD (Student's *t*-test with *unpaired* observations).

Effects of 10^{-4} M DCCD in the Tear Solution on the Response of the Transport Parameters to a Change in Tear Cl^- Concentration from 81 to 9 mM

Figure 3 shows the time course of the effect of decreasing tear Cl^- concentration in the seven experiments studied, before adding DCCD to the tear solution.

Table 4 presents the mean control values and the mean changes of the parameters at 10 min after increasing tear Cl^- from 81 to 9 mM from seven experiments. The left two columns of Table 4 present the data obtained before DCCD: I_{sc} increased by 2.2 from 4.6 μA/cm² in 81 mM Cl^-; fR_o decreased by 0.24 from 0.41; g_t decreased by 0.10 from 0.29 mS/cm² in 81 mM Cl^-; and V_o, depolarized by 13.8 from −62.9 mV in 81 mM Cl^-. The right two columns of Table 4 present the data obtained with DCCD in the tear solution: I_{sc} increased by

Table 5. Effects of adding 10^{-4} M DCCD to tear solution with Cl^--free and 10^{-5} M amphotericin B in tear solution (10 experiments)

	Control	Change in parameter
I_{SC}	4.95 ± 0.37	-0.82 ± 0.11^a
g_t	0.19 ± 0.02	0.02 ± 0.02^{ns}

Values are means ± SE. Control values obtained before DCCD. The other values are the changes at 10 min after the addition of DCCD. Units are as in Table 1.
$^a P < 0.01$; $^{ns}P > 0.05$.

4.4 from 0.79 μA/cm^2 in 81 mM Cl^-; fR_o decreased by 0.29 from 0.33; g_t decreased by 0.15 from 0.38 mS/cm^2 in 81 mM Cl^-; and V_o, depolarized by 12.4 from −48.6 mV in 81 mM Cl^-. The changes in I_{sc} (4.4 *vs.* 2.2 μA/cm^2) and g_t (0.15 *vs.* 0.10) due to the change in tear Cl^- were significantly greater in the presence of DCCD. The effects of decreasing tear Cl^- from 81 to 9 mM on fR_o and V_o were not affected by DCCD.

To pinpoint the Na^+/K^+ ATPase pump as the site of an inhibitor of the short-circuit current, Candia et al., (1974, 1984) devised a method by which the inhibitor is used in the presence of amphotericin B, in Cl^--free solutions. Amphotericin B, added to the tear solution, opens Na^+ and K^+ channels in the apical membrane of the corneal epithelium, resulting in an increase in the activity of the Na^+/K^+-ATPase and I_{sc} (Candia et al., 1974, 1984; Carrasquer et al., 1989). By removing Cl^- from the bathing media, the possible effect of the inhibitor on the NaCl cotransporter in the basolateral membrane or on the Cl^- conductance pathway in the apical membrane is eliminated. Therefore, to further support the concept that DCCD inhibits the Na^+/K^+-ATPase, the following experiments were performed.

Effect on I_{sc} and g_t upon Adding DCCD to a Concentration of 10^{-4} M in the Cornea Tear Solution in the Presence of 10^{-5} M Amphotericin B in the Tear Solution in Regular and in Cl^- Free Solutions

Table 5 shows, 10 min after the addition of DCCD, in Cl^--free solutions, there was a decrease in I_{sc} of 0.82 from 4.95 μA/cm^2 without change in g_t. These effects further support the concept that DCCD inhibits the Na^+/K^+-ATPa pump. It should be noted that the control value of I_{sc} was smaller in this group of experiments than the value observed in the past (Carrasquer et al., 1991).

The effect of DCCD on the amphotericin B-opened Na^+ and K^+ channels in the apical membrane was evaluated with the following experiments.

Table 6. Effects of changing stroma and tear K^+ concentration from 4 to 79 mM and tear Na^+ from 102 to 10 mM

	Control	Δ = 10 min	Control	Δ = 10 min
	Without DCCD		With DCCD	
	Change stroma K^+ concentration from 4 to 79 mM (5 experiments)			
I_{SC}	5.42 ± 0.58	-1.86 ± 0.22^a	4.44 ± 0.69	-1.84 ± 0.21^a
g_t	0.19 ± 0.03	0.06 ± 0.01^a	0.25 ± 0.03	0.02 ± 0.01^{ns}
	Change tear K^+ concentration from 4 to 79 mM (5 experiments)			
I_{SC}	4.12 ± 0.30	1.75 ± 0.10^a	2.78 ± 0.12	1.90 ± 0.30^a
g_t	0.22 ± 0.03	-0.02 ± 0.02^{ns}	0.20 ± 0.04	0.02 ± 0.02^{ns}
	Change tear Na^+ concentration from 102 to 10 mM (5 experiments)			
I_{SC}	5.86 ± 0.70	-4.12 ± 0.63^a	3.08 ± 0.23	-3.48 ± 0.47^a
g_t	0.20 ± 0.02	0.01 ± 0.02^{ns}	0.30 ± 0.03	-0.06 ± 0.02^b

Cl^--free and 10^{-5} M amphotericin B in tear solution. Without and with 10^{-4} M DCCD in the tear solution (5 experiments). Control values obtained before the change in K^+ concentration. The other values are the changes obtained 10 min after the change in K^+ or Na^+ concentration. Symbols and units as in Table 1.
$^a P < 0.01$; $^bP < 0.05$; $^{ns}P > 0.05$.

Effects of 10^{-4} M DCCD in the Tear Solution on the Response of I_{sc} and g_t due to a Change in K^+ Concentration in the Tear and Stroma Solutions and in Na^+ Concentration in the Tear Solution in Cl^- Free Solutions and 10^{-5} M Amphotericin B in the Tear Solution

Table 6 shows that, with an increase in K^+ concentration in stroma solution, I_{sc} decreased by 1.86 from 5.42 μA/cm^2 without DCCD and by 1.84 from 4.44 μA/cm^2 with DCCD. The increase in stroma K^+ concentration gave an increase in g_t of 0.06 from 0.19 mS/cm^2 without DCCD, without effect on g_t in the presence of DCCD. One should note the lack of effect of DCCD on the response to a change in stroma K^+ concentration (*see* Discussion). The decrease of 1.86 was significantly smaller than the decrease of 5.2 μA/cm^2 ($P < 0.01$) observed when the stroma K^+ concentration was increased in control conditions.

The Effect of Increasing K^+ Concentration in Tear Solution on I_{sc} and g_t Were not Affected by DCCD

The effects of decreasing Na^+ concentration in tear solution on I_{sc} were not statistically different without or with DCCD, −4.12 *vs.* −3.48. The decrease in Na^+ concentration in tear solution gave a decrease in g_t of 0.06 from 0.30 mS/cm^2 in the presence of DCCD. The decrease in Na^+ concentration did not affect the conductance in the absence of DCCD. Apparently, DCCD in-

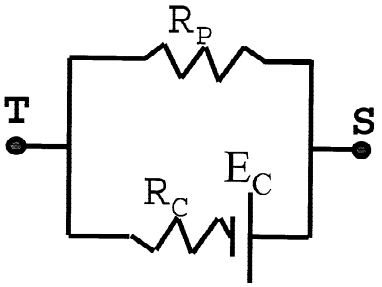

Fig. 4. Equivalent circuit across the frog cornea epithelium. E_c is the transepithelial EMF; R_c, the transcellular resistance; R_p, the resistance of the paracellular pathway. *T* and *S* refer to the tear and stroma side, respectively.

creased the sensitivity of the Na^+ conductance in the apical membrane, induced by amphotericin B.

Discussion

The main effects of DCCD were a decrease in I_{sc}, a depolarization of V_o and a decrease in fR_o. The four major pathways that contribute to the I_{sc} in the cornea epithelium are the electroneutral NaCl cotransporter in the basolateral membrane (Zadunaisky, 1972; Frizzel, Field & Schultz, 1979; Candia, 1982; Reus et al., 1983; Nagel & Carrasquer, 1989) and three electroconductive pathways, namely, the Na^+/K^+ ATPase and the K^+ conductance in the basolateral membrane (Candia, Bentley & Cook, 1974; Carrasquer et al., 1985, 1987) and the Cl^- conductance in the apical membrane (Nagel & Reinach, 1980; Reus et al., 1983; Nagel & Carrasquer, 1989). It should be noted that, with 4 mM (or greater) K^+ solutions, the Na^+/K^+ ATPase conductance is much smaller than the K^+ conductance (Carrasquer et al., 1985, 1987). An inhibition of any of the four pathways by DCCD could have been responsible for the decrease in I_{sc}. The simultaneous depolarization of V_o and a small but significant change in g_t suggest that DCCD affected one or more of the three conductive pathways: the Na^+/K^+ ATPase, the K^+ conductance and/or the Cl^- conductance. The significant decrease in fR_o by DCCD could be explained by an increase in the basolateral membrane resistance, a decrease in the apical membrane resistance or both. The combination of both effects in the apical and basolateral membranes is supported by the fact that the change in conductance was minimal. Under short-circuit conditions (*see* Fig. 4),

$$E_c = I_{sc} R_c \tag{1}$$

where E_c is the EMF responsible for the active transport across the cell; I_{sc} is the short circuit current; R_c is the transcellular resistance; R_p is the resistance of the paracellular pathway. E_c is equivalent to the Na^+ EMF of Ussing and Zehran (1951) in frog skin and to Nagel & Reinach (1980) E_{Cl} in the cornea. Since the change in g_t (or change in R_c) was very small, there is an indication that, at least in part, the decrease in I_{sc} must be explained by an inhibition of the Na^+/K^+-ATPase pump, which is the primary transporter responsible for E_c. This interpretation is further supported by the inhibition of I_{sc} in the presence of amphotericin B, in Cl^- free solutions.

The decrease in fR_o with small or no change in g_t was evaluated by studying K^+ conductance in the basolateral membrane (Candia, Bentley & Cook, 1974; Carrasquer et al., 1985, 1987) and the Cl^- conductance in the apical membrane (Nagel & Reinach, 1980; Reus et al., 1983; Nagel & Carrasquer, 1989) by the ion substitution technique. An increase in stroma K^+ concentration results in a decrease of the I_{sc}, an increase in g_t and a depolarization of V_o without changing the fR_o. If DCCD decreased the basolateral membrane K^+ conductance, the change in the parameters induced by an increase in stromal K^+, would be smaller with than without DCCD in the tear solution. This effect was observed in present experiments. Therefore, the data support the concept that DCCD decreases the basolateral membrane K^+ conductance, except in the presence of amphotericin B (*see below*).

In parallel to the above reasoning, the increase of I_{sc}, decrease of g_t, increase of fR_o, and depolarization of V_o when Cl^- concentration is decreased in the tear solution should be affected by DCCD, if the latter affects the Cl^- conductance. DCCD enhanced the change in the parameters induced by a decrease in the tear Cl^- concentration, except for the depolarization of V_o which was not significantly different with than without DCCD. These findings, in particular the higher increase in I_{sc} when Cl^- was increased with DCCD in the tear solution, support the concept that DCCD increases the apical membrane Cl^- conductance. The small change in g_t, with the definite decrease in fR_o by DCCD can be explained by the combination of the effects on the apical Cl^- and basolateral membrane K^+ conductances.

Of interest is the fact that, in the presence of amphotericin B in the tear solution and Cl^--free solutions, DCCD had no effect on the response of I_{sc} to an increase in the stroma K^+ concentration. This finding could be explained by a decrease in the basolateral K^+ conductance as a result of a decrease in the intracellular K^+ concentration (Carrasquer et al., 1991) induced by amphotericin B. As a matter of fact, the decrease in I_{sc} of 1.86 in amphotericin B/ Cl^--free solutions was significantly smaller ($P < 0.01$) than the decrease of 5.2 $\mu A/cm^2$ observed when the stroma K^+ concentration was increased in control conditions.

DCCD at a concentration of 10^{-4} M or even at a concentration of 10^{-3} M in the stroma solution had smaller effects than at a concentration of 10^{-4} M in the

tear solution. Two factors may be responsible for the difference: One, the thickness of the stroma layer between the stroma solution and the basolateral membrane and two, the liposolubility of DCCD. The latter facilitates the entrance into the cell across the apical membrane which is in direct contact with the tear solution.

The pH of the solution influenced the effects of DCCD on the transport parameters I_{sc}, V_o and g_t without influencing the effect of DCCD on fR_o. The decrease of I_{sc} and depolarization of V_o were smaller at tear pH 7.3 than at tear pH 8.5. Perhaps penetration of DCCD into the cell was greater at pH 8.5. While the changes were small, DCCD increased significantly g_t at pH 8.5 while it decreased significantly g_t at pH 7.3. The dual effect of decrease in K^+ and increase in Cl^- conductances may explain the results. At pH 8.5, the increase in Cl^- conductance may predominate while at pH 7.3 decrease in K^+ conductance may be the predominant factor. The lack of influence of pH on the effect of DCCD on fR_o could be similarly explained, since both conductance effects result in a decrease in fR_o.

In summary, we have shown that DCCD at a concentration of 10^{-4} M in the tear solution bathing the frog cornea epithelium reduces the short circuit current and depolarizes the intracellular potential. The effects may be explained by a decrease in the basolateral membrane K^+ conductance, in combination with a partial inhibition of the Na^+/K^+-ATPase pump located in the basolateral membrane. An increase in the apical membrane Cl^- conductance combined with the decrease in the K^+ conductance can explain the decrease in fR_o with small changes in g_t.

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