Effect of Pentachlorophenol (PCP) on Frog Cornea Epithelium (44436)

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Abstract. **Pentachlorophenol (PCP) is a toxic substance that affects many tissues adversely. Present experiments, using an *in vitro* preparation, were designed to study whether PCP affected the electrophysiological parameters of the bullfrog cornea epithelium, specifically, the Na^+/K^+ ATPase pump and the K^+ conductance located in the basolateral membrane and the Cl^- conductance located in the apical membrane. For this purpose, corneas were impaled with microelectrodes and experiments were done under short-circuit current (I_{sc}) conditions. Addition of PCP to a concentration of 5×10^{-5} *M* to the tear solution gave a marked decrease in I_{sc}; a marked depolarization of the intracellular potential, V_o; and minimal but significant decreases in the apical membrane fractional resistance, fR_o, and in the transepithelial conductance, g_t. I_{sc} experiments in Cl^--free solutions with amphotericin B in the tear solution confirm results indicating that PCP inhibits the active transepithelial transport mechanism and produces a small increase in the basolateral membrane resistance due to a decrease in the K^+ conductance.**

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Pentachlorophenol (PCP) has been used as an insecticide, herbicide, fungicide, bactericide, and antimildew agent since commercial-scale production began in 1936 (1). It has been recommended for use in the preservation of wood because, after treatment with PCP, it can be painted as natural wood (2). Exposure to PCP has been reported to produce toxic effects in workers as well as in people living in PCP-treated log homes. Serum levels in residents of PCP-treated log homes were higher (69–1340 p.p.b.) than in controls (15–75 p.p.b.) but much lower than in workers exposed to PCP (26–84,900 p.p.b.) (3). Evidence for renal tubular dysfunction and a decrease in GFR has been reported in workers exposed to PCP (4). At the cellular level, PCP has been found to interfere with electron transport, resulting in uncoupling of oxidative phosphorylation (5).

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Work done on biological membrane transport, related to present studies, shows that PCP inhibits the active transport of Na^+ in the toad skin (6) and inhibits the active Cl^- transport in the toad cornea (7). Nwoga *et al.* (8) showed that 10^{-4} *M* PCP depolarized single skeletal muscle cells of *Balanus nubilus*. They attributed this effect on an activation of a verapamil-sensitive Ca^{2+} influx pathway.

The frog cornea enables the ready use of microelectrodes and, for this reason, provides an excellent means to test the biological effects of toxic and other substances that affect epithelial transport. The cornea has a Cl^--secreting epithelium, in which Cl^- is actively transported from stroma to tear. The primary active transport is the Na^+/K^+ ATPase pump located in the basolateral membrane (9–11). Other transporters that play an important role in Cl^- secretion are the Cl^- conductance located in the apical membrane (12–14), the K^+ conductance (9–11), and the NaCl symport located in the basolateral membrane (13–17). 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 previous studies on the toad skin and toad cornea mentioned above, without the use of microelectrodes, the specific transport pathways responsible for the decrease in short-circuit current could not be determined. In present studies, using the microelectrode technique, we can assess

which mechanisms mentioned above are responsible for the decrease in short-circuit current.

Materials and Methods

Bullfrog corneas (*Rana catesbeiana*) were mounted tear side up in a lucite chamber as previously described (11, 12, 18). The tissue was supported by a copper grid with a slightly smaller radius of curvature than that of the *in vivo* cornea. The endothelium rested on the copper grid. An opening of 0.4 cm^2 connected the upper (epithelial) chamber (0.2 ml) with the lower (stroma) chamber (0.3 ml). Note that the term stroma chamber or solution is used throughout the paper with reference to chamber or solution closest to the stroma. 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 m*M*) (stroma): Na^+, 102; K^+, 4.0; 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, 1; and glucose, 10. K^+ was substituted for Na^+ in high K^+ solutions. In experiments reported in this paper, PCP was added to the tear solution to a final concentration of 5×10^{-5} *M*, which is the minimal concentration significantly affecting the cornea. Pilot experiments in which the concentrations were below 5×10^{-5} *M* showed no or minimal effects. PCP was added to the stroma solution up to a final concentration of 5×10^{-5} *M* or 5×10^{-4} *M* with no or minimal effects. The concentration 5×10^{-5} *M* is ≈ 10,000 p.p.b., which is within the range of the serum concentration found in workers exposed to PCP (3). Amphoterecin 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 in the tear solution of 8.5–8.6. Candia (19) showed that high pH in the tear solution was favorable for high I_{sc} and Cl^- fluxes. Since the pH of the solution affects the ionization of PCP, two different pHs, namely, 7.3–7.4 and 8.5–8.6, were used at the site of addition of PCP in experiments in which the effects of PCP were studied. 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 that had an input resistance of 50–70 Mohm. Corneas were short-circuited using an automatic clamp device (Biomed. Inst., Germering, FRG) 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 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}), g_t, fR_o, and V_o, 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, the opposite.

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

Results

Effect of Adding PCP to a Concentration of 5×10^{-5} *M* in the Tear Solution. Initial experiments were performed at a pH of 8.5 in tear solution and 8.5 in stroma solution. We found that PCP affected the transport parameters when added to the tear but had minimal effect when added to the stroma solution. Since the pK_a of PCP is 5.0, the difference of the nonionized PCP at the two pHs is minimal, between pH 7.3 and 8.5 (difference <0.5 m*M*). Despite this small difference, experiments were done at the two different pHs in the tear solution to rule out the effect of pH on the activity of PCP. The stroma solution pH in these experiments was maintained at 7.3.

The left panel of Figure 1 shows the effects of PCP when added to the tear solution with pH of 8.5 in the tear solution. The curves represent the mean values, from seven experiments, of I_{sc}, fR_o, g_t, and V_o plotted versus time, with zero being the time PCP was added. The right panel of Figure 1 shows the effects of PCP when added to the tear solution with pH of 7.3 in the tear solution. The effects were similar to those shown in the left panel.

Although Figure 1 shows the time course of the experiments, Table I presents numerical data of the mean control values and the mean changes of the parameters at 10 min after addition of PCP. The left two columns of Table I present the data obtained at pH 8.5 in the tear solution. I_{sc} decreased by 2.5 from 5.2 $\mu A/cm^2$ control; fR_o decreased by 0.09 from 0.39 control; g_t had a very small but significant decrease of 0.03 from 0.33 mS/cm^2 control; and V_o, depolarized by 10.5 from −48.3 mV control.

The right two columns show that the effects of PCP at pH of 7.3 were similar to those at pH 8.5 in the tear solution.

The addition of PCP to a 10-fold lower concentration in the tear solution gave similar but smaller effects except that there was no significant effect on fR_o at the lower concentration.

Effect of Adding PCP to a Concentration of 5×10^{-5} *M* in the Stroma Solution. These experiments were done at two different pHs in the stroma solution, namely, 7.3 and 8.5. The pH was kept at 8.5 in the tear solution.

Table II presents data on the effects of PCP when added

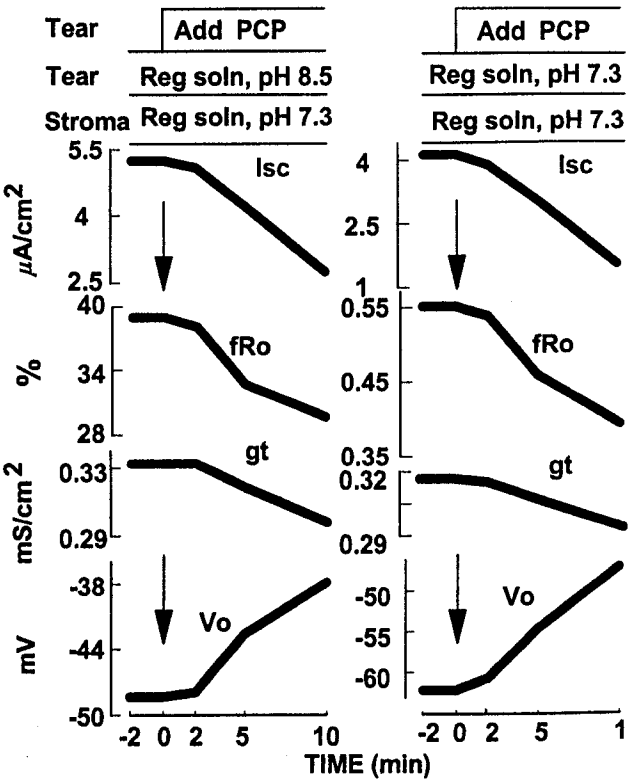

Figure 1. Effect of 5×10^{-5} *M* PCP in the tear solution. The left panel represents pH 8.5 and the right panel pH 7.3 in the tear solution. The stroma pH was 7.3 in both cases. Values are means from seven experiments with tear pH 8.5 and six experiments with tear pH 7.3. Short-circuit current, I_{sc}, in μA/cm²; apical membrane fractional resistance, fR_o, unitless; transepithelial conductance, g_t, in mS/cm²; intracellular potential, V_o, in mV; all parameters are plotted versus time. Zero time is when PCP was added.

to a concentration of 5×10^{-5} *M* in the stroma solution. There were no effects on any of the four bioelectrical parameters when the pH of the stroma solution was 7.3 (left two columns). There was a minimal decrease in I_{sc} and a small depolarization of V_o when the pH of the stroma solution was 8.5. The other two parameters, fR_o and g_t, were not affected.

One of the major effects of PCP, when added to the tear solution, is the decrease in I_{sc}. The transport pathways that affect the I_{sc} in the corneal epithelium are the Na^+/K^+-ATPase, NaCl cotransporter, and K^+ conductance in the basolateral membrane and the Cl^- conductance pathway in the apical membrane (See references above). A decrease in fR_o concomitant with a decrease in g_t suggests that the decrease in conductance by PCP must be in the basolateral membrane. Therefore, PCP must have decreased the K^+ conductance. Consequently, the effect of PCP on the K^+ partial conductance in the basolateral membrane was evaluated by the ion substitution method.

Effects of 5×10^{-5} *M* PCP in the Tear Solution on the Response of the Transport Parameters to a Change in Stroma Solution K^+ Concentration from 4 to 79 m*M*. In these experiments, the pH in the tear solution was 8.5 and in the stroma solution, 7.3. Figure 2 shows the effect of changing stroma solution K^+ concentration. Results without PCP are on the left side and with PCP on the right side of the figure.

Table III presents the mean control values and the mean changes of the parameters at 6 min after increasing stroma solution K^+ from 4 to 79 m*M*. Without PCP, I_{sc} decreased by 6.0 from 4.4 μA/cm²; fR_o did not change; g_t increased by 0.15 from 0.48 mS/cm²; and V_o, depolarized by 35.2 from –73.5 mV. With PCP in the tear solution, I_{sc} decreased by 1.6 from 0.8 μA/cm²; fR_o did not change; g_t increased slightly, but significantly, by 0.01 from 0.25 mS/cm²; and V_o, depolarized by 17.4 from –43.0 mV. The effects of increasing stroma solution K^+ from 4 to 79 m*M* on I_{sc}, g_t and V_o were significantly decreased in the presence of PCP in the tear solution when compared to the change in stroma solution K^+ in the absence of the poison.

Addition of amphotericin B to the tear solution resulted in an increase in the activity of the Na^+/K^+-ATPase as a result of the opening of Na^+ and K^+ channels in the apical membrane of the corneal epithelium (9, 20, 21). This fact makes the effect of an inhibitor of the pump in the presence of amphotericin B more evident than in its absence, particularly if the inhibitor is added in Cl^--free solutions. The possible effect of the inhibitor on the NaCl cotransporter in the basolateral membrane or on the Cl^- conductance path-

Table I. Effects of Adding 5×10^{-5} *M* PCP 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 10 min	Control Tear pH 7.3	Change in parameter 10 min
	(7 expts.)		(6 expts.)	
I_s	5.24 ± 1.05	–2.49 ± 0.45[a]	4.1 ± 0.3	–2.6 ± 0.24[a]
fR_o	0.39 ± 0.04	–0.09 ± 0.03[b]	0.55 ± 0.08	–0.16 ± 0.04[b]
g_t	0.33 ± 0.03	–0.03 ± 0.006[a]	0.30 ± 0.03	–0.02 ± 0.004[a]
V_o	–48.3 ± 2.8	10.5 ± 1.0[a]	–62.1 ± 3.7	14.9 ± 1.9[a]

Note. Values are means ± SE. Control values obtained before the addition of PCP. The other values are the changes obtained 10 min after the addition of PCP. Units are: I_{sc}, μA/cm²; g_t, mS/cm²; fR_o, unitless; V_o, mV.
[a] $P < 0.01$.
[b] $P < 0.05$.

Table II. Effects of Adding 5×10^{-5} *M* PCP to Stroma Solution with pH 8.5 in Tear Solution and with Two Different pHs of 7.3 and 8.5 in Stroma Solution

	Control Stroma pH 7.3	Change in parameter 10 min	Control Stroma pH 8.5	Change in parameter 10 min
	(5 expts.)		(6 expts.)	
I_s	5.01 ± 1.2	-0.1 ± 0.1^{ns}	5.3 ± 0.6	-0.9 ± 0.2^{a}
fR_o	0.44 ± 0.05	-0.00 ± 0.02^{ns}	0.54 ± 0.04	-0.02 ± 0.008^{ns}
g_t	0.30 ± 0.03	0.00 ± 0.02^{ns}	0.32 ± 0.03	0.01 ± 0.003^{ns}
V_o	−49.4 ± 4.7	1.9 ± 0.9^{ns}	−59.7 ± 3.0	3.0 ± 0.8^{b}

Note. Values are means ± SE. Symbols and units as in Table I.
ns $P > 0.05$.

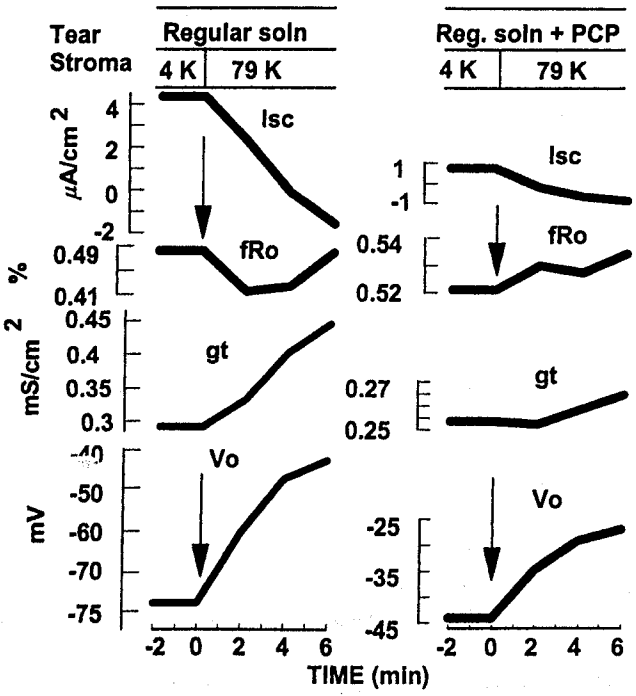

Figure 2. Effect of changing the concentration of K^+ in the stroma solution from 4 to 79 m*M*. Values are means from eight experiments before PCP (left panel) and eight experiments with 5×10^{-5} *M* PCP in the tear solution (right panel). Symbols as in Figure 1. Zero time when K^+ concentration was changed.

way in the apical membrane is eliminated in Cl^--free solutions. To further support the concept that there is an effect of PCP on the Na^+/K^+-ATPase and the K^+ pathways, the following experiments were performed.

Effect on I_{sc} and g_t Upon Adding PCP to a Concentration of 5×10^{-5} *M* in the Cornea Tear Solution in the Presence of 10^{-5} *M* Amphotericin B in the Tear Solution in Regular and Cl^--Free Solutions. Table IV shows, 10 min after the addition of PCP in regular solutions, a decrease of I_{sc} by 2.9 from 7.2 μA/cm² and a decrease of g_t by 0.03 from 0.28 mS/cm². In Cl^--free solutions, PCP gave a decrease in I_{sc} of 1.8 from 5.1 μA/cm² and no change in g_t.

To evaluate the K^+ conductance further, the following experiment was performed.

Effects of 5×10^{-5} *M* PCP in the Tear Solution on the Response of I_{sc} and g_t due to a Change in K^+ Concentration from 4 to 79 m*M* in Cl^--free Solutions and 10^{-5} *M* Amphotericin B in the Tear Solution. Table V shows that, with an increase in K^+ concentration in the stroma solution, I_{sc} decreased by 3.2 from 6.0 μA/cm² without PCP and by 1.1 from 2.2 μA/cm² with PCP. The decrease of 1.1 μA/cm² with PCP was significantly different from the decrease of 3.2 μA/cm² without PCP. The conductance, g_t, was not affected by the change in K^+ concentration in the stroma solution with or without PCP.

Discussion

Let us first examine the decrease in I_{sc} induced by PCP. The four major pathways that contribute to I_{sc} in the cornea epithelium are the electroneutral NaCl cotransporter in the basolateral membrane (13–17) and three electroconductive pathways, namely, the Na^+/K^+ ATPase and the K^+ conductance in the basolateral membrane (9–11) and the Cl^- conductance in the apical membrane (12–14). An inhibition of any of the four pathways by PCP could have been responsible for the decrease in I_{sc}. The simultaneous depolarization of V_o and the decrease in g_t suggest that PCP affected one or more of the three pathways: the Na^+/K^+ ATPase, the K^+ conductance and/or the Cl^- conductance. Since PCP also induced a decrease in fR_o, the decrease in g_t must have been on the basolateral membrane conductances, particularly the K^+ conductance, but not on the Cl^- conductance located in the apical membrane or, at best, to a lesser extent.

Under short-circuit current conditions (see Fig. 3),

$$E_c = I_{sc} R_c \quad (1)$$

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; and R_p is the resistance of the paracellular pathway. E_c is equivalent to the Na^+ EMF of Ussing and Zehran in frog skin (22) and to Nagel and Reinach E_{cl} in the cornea (12). Since the decrease in g_t (or increase in R_c) was very small, one can assume that most of the decrease in I_{sc} is explained by an inhibition of the Na^+/K^+-ATPase pump, which is mainly responsible for E_c. This is further supported by the fact that the addition of PCP to the stroma solution at pH 8.5 resulted in a small but

Table III. Effects of Changing Stroma K^+ Concentration from 4 to 79 m*M* without and with 5×10^{-5} *M* PCP in the Tear Solution (8 expts.) with pH 8.5 in Tear Solution and 7.3 in Stroma Solution

	Control	Δ at 6 min	Control	Δ at 6 min
	Without PCP		With PCP	
I_{sc}	4.4 ± 0.4	−6.0 ± 0.4[a]	0.8 ± 0.2	−1.6 ± 0.2[a]
fR_o	0.48 ± 0.04	−0.00 ± 0.01[ns]	0.50 ± 0.04	0.03 ± 0.03[ns]
g_t	0.29 ± 0.03	0.15 ± 0.02[a]	0.25 ± 0.03	0.01 ± 0.004[b]
V_o	−73.5 ± 3.7	35.2 ± 2.9[a]	−43.0 ± 5.3	17.4 ± 2.5[a]

Note. Control values obtained before the change in K^+ concentration. The other values are the changes obtained 6 min after the change in K^+ concentration. Symbols and units as in Tables I and II.

Table IV. Effects of Adding 5×10^{-5} *M* PCP to Tear Solution with 10^{-5} *M* Amphotericin B in the Tear Solution in Regular and Cl^--Free Solutions

	Control	Δ at 10 min	Control	Δ at 10 min
	Regular solutions (5 expts.)		Cl^--free solutions (6 expts.)	
I_s	7.2 ± 0.6	−2.90 ± 0.41[a]	5.1 ± 0.3	−1.8 ± 0.2[a*]
g_t	0.28 ± 0.01	−0.03 ± 0.005[a]	0.24 ± 0.02	−0.03 ± 0.01[ns]

Note. Values are means ± SE. Control values obtained before the addition of PCP. The other values are the changes obtained 10 min after the addition of PCP. Symbols and units as in Tables I and II.

Table V. Effects of Changing Stroma K^+ concentration from 4 to 79 m*M*, in Cl^--Free Solutions and with 10^{-5} *M* Amphotericin B in the Tear Solution Without and With 5×10^{-5} *M* PCP in the Tear Solution

	Control	Δ at 10 min	Control	Δ at 10 min
	Without PCP		With PCP	
	Change stroma K^+ conc. from 4 to 79 m*M* (8 expts.)			
I_{sc}	6.0 ± 0.6	−3.2 ± 0.7[a]	2.2 ± 0.2	−1.1 ± 0.2[a]
g_t	0.26 ± 0.01	0.0 ± 0.02[ns]	0.29 ± 0.02	0.03 ± 0.01[ns]

Note. Control values obtained before the change in K^+ concentration. The other values are the changes obtained 10 min after the change in K^+ concentration. Symbols and units as in Tables I and II.

significant decrease in I_{sc} and a small depolarization of V_o, without any effect on fR_o or g_t (see Eq. 1).

Since in 4 m*M* K^+ solutions, the dominant conductance in the basolateral membrane is the K^+ conductance (9–11), we considered the possibility that PCP decreased the basolateral membrane K^+ conductance. This possibility was evaluated using the ion substitution technique. If PCP decreased the basolateral membrane K^+ conductance, the depolarization of V_o induced by an increase in the stroma solution K^+ concentration (10, 11) would be smaller with than without PCP in the tear solution. This effect was observed in present experiments. Therefore, these data indicate that PCP decreased the basolateral membrane K^+ conductance. As a consequence, the depolarization of V_o by PCP can be explained by a decrease in the K^+ conductance in addition to the inhibitory effect on the pump.

Experiments in Cl^--free solutions, with amphotericin B in the tear solution, further supported the inhibition of the pump and the decrease in K^+ conductance due to PCP. Under these conditions, I_{sc} was significantly decreased by PCP as it was in Cl^- solutions. Since the major available pathway for I_{sc} is the pump, the latter must have been inhibited. Certainly the inhibition was not complete since

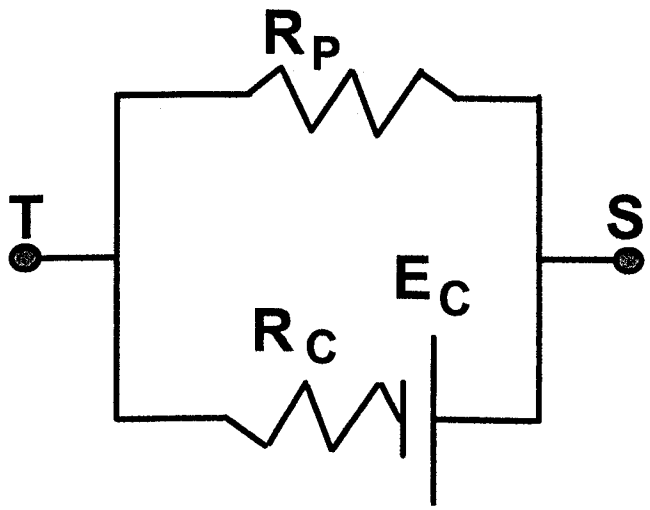

Figure 3. Equivalent circuit across the frog cornea epithelium. E_C is the transepithelial EMF; R_c, the transcellular resistance; R_P, the resistance of the paracellular pathway. *T* and *S* refer to the tear and stroma side, respectively.

PCP inhibited I_{sc} by 35%–40%. Also part of the decrease in I_{sc} may be due to the decrease in K^+ conductance.

With regard to the K^+ conductance, we noted that amphotericin B opens K^+ channels in the apical membrane.

The decrease in I_{sc} in going from 4 to 79 mM K^+ in the stroma solution was less with than without PCP. Since in this experiment only K^+ conduction is involved, the effect of PCP on I_{sc} implies that PCP affected the conductance of the K^+ pathway.

We noted that the effect of PCP at a concentration of 5×10^{-5} M or higher in the stroma solution was much smaller than when 5×10^{-5} M was used in the tear solution. The stroma presents a barrier of about 10 μm between the bathing solution and the basolateral membrane, whereas the tear solution is in contact with the apical membrane. The basal layers of the epithelium plus the endothelium on the stroma side may add to the barrier to PCP placed in the stroma solution. We further noted that the concentration 5×10^{-5} M is ≈ 10,000 p.p.b., which is within the range of the serum concentration found in workers exposed to PCP (3).

In summary, PCP at a concentration of 5×10^{-5} M in the tear solution reduces the short-circuit current and depolarizes the intracellular potential. The effects are explained by a decrease in ion transport by the Na^+/K^+-ATPase located in the basolateral membrane, combined with a decrease in the basolateral membrane K^+ conductance. PCP at a concentration of 5×10^{-5} M or even 10^{-4} M in the stroma solution had minimal or insignificant effects. For a range of pH from 7.3 to 8.5, the tear pH did not influence the effect of PCP on the electrophysiological parameters.

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