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The Stimulation of Chloride Transport by Prostaglandins and their Interaction with Epinephrine, Theophylline, and Cyclic AMP in the Corneal Epithelium

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Summary. Prostaglandins (E_1, E_2 and $F_{2\alpha}$) stimulated the chloride transport of the frog corneal epithelium with maximal effects at 10^{-5} M in the aqueous side. This stimulation does not occur in Cl-free solutions and the net ^{36}Cl flux increased proportionally to the short-circuit current. Polyphloretin phosphate (PPP) and diphloretin phosphate (DPP) inhibited the response if added within 3 min before PGE_1. The maximal response to epinephrine 10^{-5} M and dibutyryl cyclic AMP 10^{-3} M was not changed by further addition of prostaglandins, but these drugs produced their full effect when administered at the peak of the response of prostaglandins. The maximal response to theophylline 10^{-5} M was increased by PGE_1. PPP and DPP did not modify the response to epinephrine. Prostaglandin stimulation of the chloride transport was accompanied by increased light transmission through partially opaque corneas. The known release of prostaglandins in the aqueous humor can be associated to a direct action on the corneal epithelium manifested in the activation described herein.

The transparency of the cornea is dependent on the state of hydration of the stroma. The two principal cell layers of this tissue, the epithelium in front, and the endothelium inside, contribute by their permeability properties and, by the presence of ionic pumps, to the control of the state of hydration of the stroma (Maurice, 1969; Zadunaisky, 1973).

The epithelium of the frog cornea transports chloride ions out into the tear side (Zadunaisky, 1966), and this mechanism has been shown to be

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able to dehydrate and clear partially opaque, swollen corneas (Zadunaisky& Lande, 1971). More recently, Chalfie, Neufeld and Zadunaisky (1972) have found that epinephrine is a potent stimulant of the chloride transport of the frog corneal epithelium. The stimulation is also obtained with derivatives of cyclic AMP and with inhibitors of phosphodiesterase such as theophylline and aminophylline. Therefore, on the basis of the known effects of epinephrine on adenyl cyclase in other tissues it was concluded that epinephrine acted by means of an increase in the tissue concentration of the "second messenger", cyclic AMP (Robison, Butcher & Sutherland, 1971).

In the rabbit cornea, the application of epinephrine produces a remarkable stimulation of chloride transport outwards, in the direction from aqueous to tears, which is similar to the one in the frog cornea (Klyce, Neufeld & Zadunaisky, 1973; Zadunaisky, Lande, Chalfie & Neufeld, 1973). In this case, the cyclic AMP content of incubated corneas, stimulated with epinephrine and theophylline, increased to twice the control values. The presence of this chloride pump in the rabbit cornea, together with a well-known sodium pump performing inwards, demonstrates that no real difference exists between amphibians and mammalians with respect to corneal functions. On the other hand, the stimulation of the chloride transport by the increase in the cyclic AMP content of the epithelial cells is similar to the behavior of the rabbit intestine (Field, 1971).

Prostaglandins have been explored in ocular function for quite some time. Irin, the substance described by Ambache (1957; *see also* Ambache, Kavanagh & Whiting, 1965) extracted from the iris and with action on smooth muscle was identified as a prostaglandin (Anggard & Samuelson, 1964). The increase in intraocular pressure produced by irritating agents is prevented by polyphloretin phosphate (Cole, 1961; Bethel & Eakins, 1971) and it has been shown to be due to the release of prostaglandins into the aqueous humor (Beitch & Eakins, 1969; Eakins *et al.*, 1972). The prostaglandins have also been shown to be transported out of the eye by an active mechanism (Bito, 1973).

This paper describes the actions of PGE_1, E_2, and $F_{2\alpha}$ on the electrical and transport properties of the frog corneal epithelium. The findings indicate that these compounds have a stimulatory action on the chloride transport of the corneal epithelium, and interact with epinephrine, theophylline, and cyclic AMP. Their action is most probably mediated through the cyclic AMP system. Experiments with the prostaglandin inhibitors polyphloretin phosphate (PPP) and diphloretin phosphate (DPP) are also described.

Materials and Methods

Corneas of *Rana catesbeiana* were excised and mounted as membranes in Lucite chambers which permitted continuous control of the bathing fluids on both sides during the recording of electrical parameters. Methods for determining the short-circuit current (SCC) and potential difference (p.d.) between the endothelial and epithelial sides of the cornea have been described by Zadunaisky (1966). Short-circuit current was monitored continuously and the potential difference was recorded at different intervals. These values were used to calculate resistance. The volume of Conway-Ringer's solution in each chamber was 3 ml. The fluid level on the endothelial side was adjusted to 0.5 cm higher than on the epithelial side, in order to maintain the normal corneal conformation by this small pressure difference. Agar-Ringer's bridges were positioned and the short-circuit current was allowed to stabilize approximately 1 hr. The bathing fluid was aerated and circulated so that drugs introduced to the system were quickly diluted to predetermined concentration and brought to the appropriate corneal surface. Unless otherwise noted, prostaglandins were introduced on the endothelial side of the isolated cornea. Several dilutions of PGE_1, E_2, and $F_{2\alpha}$ were made to determine the SCC and p.d. responses of the cornea to various concentrations. Subsequently, PGE_1 was used, at a concentration of 1×10^{-5} M.

In a series of six experiments to determine whether chloride was involved in the response to prostaglandins, the following procedure was used. PGE_1 was administered first in chloride-Ringer's, and a response observed; this solution was then replaced with chloride-free Ringer's solution, osmotically balanced with sulfate ion, and after equilibration another dose of PGE_1 was administered. To determine that the cornea was still physiologically responsive, the bathing fluid was replaced with normal chloride-Ringer's solution again and after equilibrium, a final dose of PGE_1 was given.

The fluxes of ^{36}Cl were determined by methods previously described (Zadunaisky, 1966; Chalfie *et al.*, 1972). The apparatus and techniques for measurement of transparency of the frog corneas were those of Zadunaisky and Lande (1971).

The effect of PPP and DPP on the response of the SCC to PGE_1 was investigated as follows: a normal response to PGE_1 was recorded, and the chambers washed out with fresh Ringer's solution. DPP was then applied to both sides of the cornea in a final concentration of 0.1 mg/ml, after which PGE_1 was tested again. The interaction between DPP and epinephrine was tested by treating both sides of the cornea with DPP (0.1 mg/ml) prior to applying 1×10^{-5} M epinephrine to the endothelial side.

Three types of epinephrine experiments were conducted. First, epinephrine was administered to the endothelial side of the cornea while it was responding maximally to PGE_1. In each, the response of the SCC to epinephrine alone was tested before and after the PGE_1-epinephrine interaction. The order was reversed in a second series of experiments in which PGE_1 was administered about 3 min prior to the peak response to epinephrine. This took into account the latent period characteristic of the response to PG. Finally, in a few experiments PGE_1 was given after the peak of the SCC response to epinephrine. In the prostaglandin-theophylline experiments a dose of 3×10^{-5} M theophylline was given on the endothelial side of the cornea. The response was followed with a washout and re-equilibration of the SCC, after which 1×10^{-5} M PGE_1 was given. At the peak of the PGE_1 response, a second dose of 3×10^{-5} M theophylline was given. In two additional experiments, the order was reversed.

Two types of PGE_1-dibutytyl cyclic AMP (Db cAMP) experiments were carried out; in all of them, Db cAMP was added to both sides of the cornea simultaneously (1×10^{-3} M). In the first series, the cornea received Db cAMP then PGE_1 at the peak of the Db cAMP response, while in the other experiments the order of administration was reversed.

PGE_1 and PGE_2 were diluted with Conway-Ringer's solution from alcoholic stock solutions, while the trimethamine salt of $PGF_{2\alpha}$ was dissolved directly in Conway-Ringer's solution.

L-epinephrine bitartrate, theophylline and N^6, $O^{2'}$-dibutyryl adenosine 3′:5′-monophosphoric acid (Db cAMP) used in this study were products of Sigma Chemical Co., St. Louis, Missouri.

Prostaglandins were obtained from The Upjohn Co., Kalamazoo, Michigan, polyphloretin phosphate and the dimer polyphloretin phosphate were products of A.B. Leo Laboratories, Helsingborg, Sweden.

Results

Response of the Frog Cornea to Prostaglandins

The introduction of prostaglandins to the fluid bathing the endothelial side of the frog cornea caused a consistent and sustained increase in the potential difference (p.d.). Moreover, this response was completely reversible upon washing out the prostaglandin and could be duplicated upon repeated doses (Fig. 1). PGE_1, E_2 and $F_{2\alpha}$ produced approximately the same maximal increase on the short-circuit current (Table 1). However, the dose necessary to achieve this maximum was 1×10^{-6} M for PGE_2, 1×10^{-5} M for PGE_1 and 2×10^{-5} M for $PGF_{2\alpha}$. Though PGE_2 produced the maximal response with 10 times less concentration than PGE_1 and 20 times less than $PGF_{2\alpha}$, further increase of the dose of PGE_2 produced a decrease in the response. When applied to the epithelial (tear) side of the cornea, PGE_2, at concentrations of 1, 2 and 5×10^{-5} M, elicited a response qualitatively similar to that resulting from endothelial (aqueous side) administration; however, the epithelial route was much less effective (Table 2).

Corneal Response to Prostaglandins Under Chloride-Free Conditions

In the absence of chloride, the short-circuit current equilibrated at a low value 2.9 ± 0.8 μamps/cm^2 [n = 6] compared with 19.2 ± 3.1 μamps/cm^2

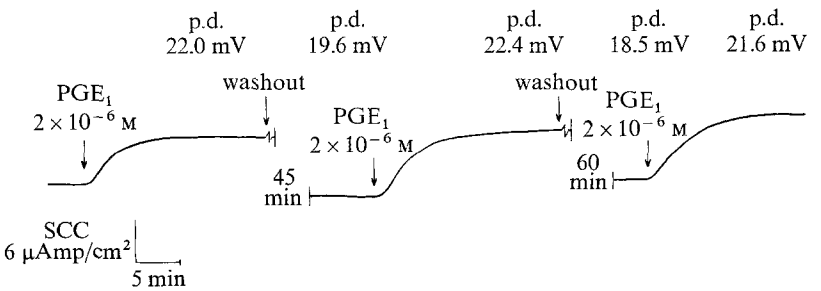

Fig. 1. Repeated effects of prostaglandin E_1 on the short-circuit current of isolated frog cornea

Table 1. Dose-dependency of the response of the frog cornea to prostaglandins

		5×10^{-7}	1×10^{-6}	2×10^{-6}	5×10^{-6}	1×10^{-5}	2×10^{-5}
PGE_1	SCC		15.8±4.7	35.7±6.6	43.3±9.0	52.8±8.0	38.5±6.1
	p.d.		3.1±2.9	16.6±2.8	19.1±3.9	12.9±1.9	11.2±3.2
	R		−1.6±3.2	−20.2±3.9	−16.8±3.7	−25.1±3.1	−18.6±5.0
			[6]	[10]	[7]	[11]	[8]
PGE_2	SCC	30.1±6.4	55.1±9.1	42.6±9.2	46.2±11.9	33.8±9.5	34.3±10.1
	p.d.	5.1±1.3	8.5±1.9	7.5±1.0	9.5±4.1	10.8±4.1	2.6±2.5
	R	−17.1±3.7	−28.9±4.0	−23.2±4.4	−24.4±4.9	−26.2±5.4	24.5±6.9
		[6]	[6]	[6]	[5]	[6]	[6]
$PGF_{2\alpha}$	SCC		19.2±15.9	18.1±7.1	37.7±8.1	43.1±23.4	50.7±19.8
	p.d.		8.7±4.0	24.7±6.2	33.3±12.1	18.5±8.8	29.3±8.4
	R		−8.1±8.0	−8.1±11.4	−18.4±18.2	−10.3±3.5	11.2±6.5
			[4]	[5]	[4]	[4]	[5]

Fractional changes in short-circuit current (SCC), potential difference (p.d.), and resistance (*R*) are expressed as percent change over the control level. Means ± SE. Number of experiments in brackets.

Table 2. Effect of prostaglandin E_2 applied on the epithelial side of the isolated frog cornea

Concentration of PGE_2 (moles)	1×10^{-5}	2×10^{-5}	5×10^{-5}
SCC	6.3±1.6	14.5±5.8	29.0±3.7
p.d.	0.7±2.3	1.8±3.3	5.5±2.1
R	−4.3±1.7	−17.7±2.4	−18.0±2.0

Changes in short-circuit current (SCC), potential difference (p.d.), and trans-membrane resistance (*R*) are indicated as percent of the original stabilization values. Mean ± SE [n = 6].

[n = 6] in the presence of chloride. Under chloride-free conditions, PGE_1 failed to evoke any change in SCC. When this bathing fluid was replaced with complete Conway-Ringer's solution, the SCC increased to normal (18.5 ± 2.9 μamps/cm² [n = 6] and the cornea immediately regained its ability to respond to PGE_1 in the normal manner (Fig. 2).

The Effect of DPP on the Response of the SCC to PGE_1

In the presence of DPP, the response to PGE_1 was markedly reduced; only a 12.5% increase in SCC, in contrast with a control value of 53%. In

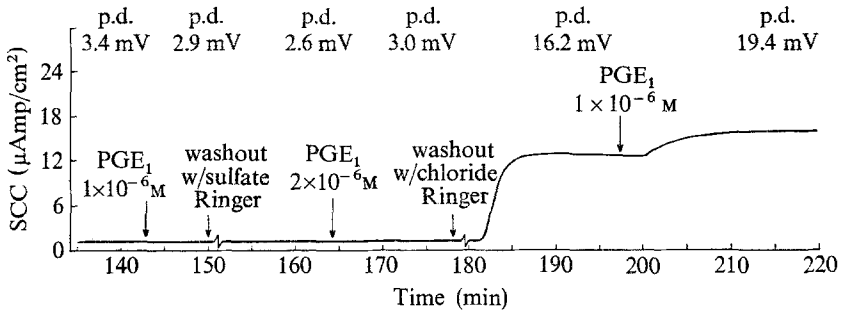

Fig. 2. Lack of increase in short-circuit current of frog cornea in absence of chloride ions in the bathing solutions. After stabilization of the current in Cl-free solutions, PGE_1 has no action; after washout with Cl-free solutions again there is no response. But when chloride-containing solution is used, there is a response

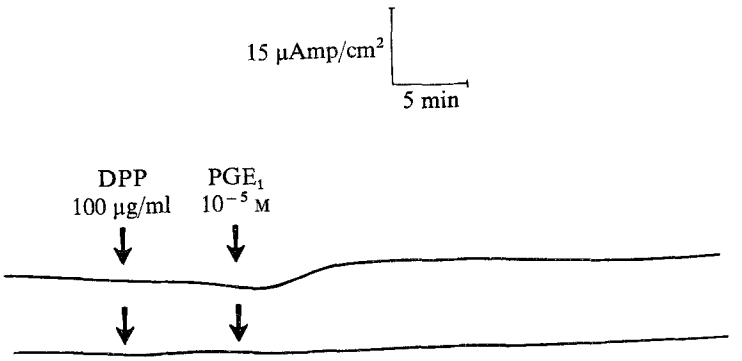

Fig. 3. Inhibitory effect of DPP on the response of the short-circuit current to PGE_1. In two companion corneas the addition of DPP completely inhibits the response to PGE_1 (bottom curve) or produces partial inhibition (upper trace)

order for DPP to be effective, it was necessary to give it within 3 min before the addition of PGE_1 (Fig. 3, Table 3). DPP alone had no effect upon the SCC.

Prostaglandin and Epinephrine

When 10^{-5} M epinephrine was administered while the cornea was responding maximally to 10^{-5} M PGE_1, the SCC increased further to 134% above the stabilization level (Fig. 4, Table 3). This response was not significantly different from the increase to epinephrine alone (Table 3).

In the reverse situation, however, where PGE_1 was given just before the peak of a response to epinephrine, no further increase was observed, (Table 3). When PGE_1 was administered during the declining phase of

Table 3. Interactions between prostaglandin E_1 (PGE_1) and epinephrine, Db cAMP and theophylline

Treatment	Change in SCC[a] (% increase ± SE)	No. of Exp.
PGE_1 (10^{-5} M)	53.4 ± 5.4	33
PGE_1 after DPP (0.1 µg/ml)	12.5 ± 5.1	9
Epinephrine (10^{-5} M)	155.0 ± 17.6	14
Epinephrine after DPP	166.3 ± 34.6	3
PGE_1 + epinephrine at PGE_1 peak	133.8 ± 28.0	7
Epinephrine + PGE_1 at epinephrine peak	139.0 ± 24.0	8
Theophylline (3×10^{-5} M)	86.3 ± 9.3	8
PGE_1 + theophylline at PGE_1 peak	114.2 ± 18.6	8
Db cAMP (10^{-3} M)	107.3 ± 20.7	5
Db cAMP + PGE_1 at Db cAMP peak	118.7 ± 29.3	4
PGE_1 + Db cAMP at PGE_1 peak	110.1 ± 22.9	4

[a] In cases of drugs added sequentially, the increases for the second drug were measured with respect to the equilibrium level after the first drug.

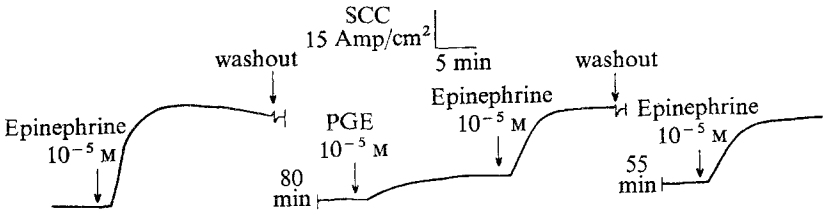

Fig. 4. Interaction of PGE_1 with epinephrine. The first trace is the full response to epinephrine of a frog cornea; the second trace shows that the full response of epinephrine is obtained after PGE_1, and the last shows again the full response to epinephrine

the response of the SCC to epinephrine, a slight effect was noted. The intensity of this effect appeared to increase as the cornea recovered from epinephrine (Fig. 5). No inhibition was observed in the response to epinephrine after treatment with DPP (Fig. 6, Table 3).

Prostaglandin and Theophylline

Theophylline produced a sustained elevation in SCC, taking longer to reach a peak than after prostaglandin. In two experiments, addition of PGE_1 at the height of the response to theophylline further increased the SCC from 92 to 130% and from 83 to 117% over the original stabilization value. When theophylline was given while the cornea was responding maximally to PGE_1, the SCC doubled (Table 3).

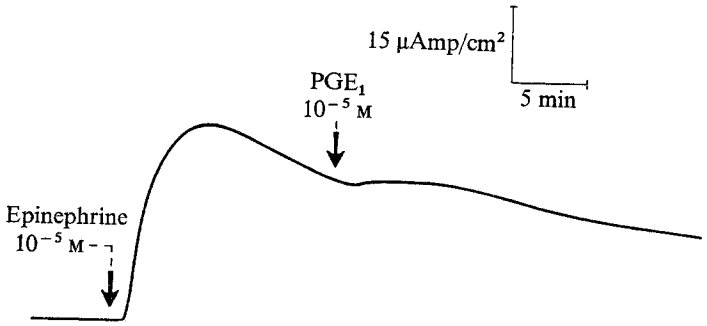

Fig. 5. Response to PGE_1 after epinephrine. This initial response is obtained only after the peak of the effect of epinephrine. Otherwise, there is no response to PGE_1 during the maximal effect of epinephrine

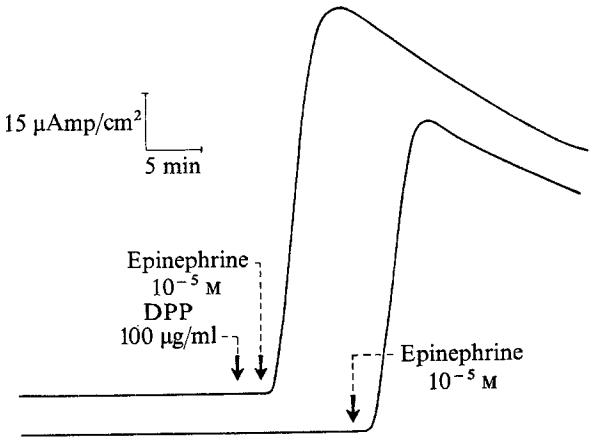

Fig. 6. Lack of inhibition of DPP of the response to epinephrine in two companion corneas of the frog. The addition of DPP to the bathing solution of one cornea does not change quantitatively the response to epinephrine

Prostaglandin and Db cAMP

After a 5-min latent period, Db cAMP caused a prolonged and gradual rise in the SCC, which reached a maximum of about twice the original value in about 2 hr. When PGE_1 was added at the peak of the response to Db cAMP, there ensured an additional small rise in SCC, considerably less than the effect of PGE_1 alone. Following the usual latent period, a slight and transient decrease in SCC preceded the subsequent rise. When the order was reversed and Db cAMP was given at the peak of the response to PGE_1, a similar diphasic effect was observed; i.e., the SCC dropped slightly during the next 10 min followed by a response qualitatively similar to that of Db cAMP alone.

Table 4. Chloride fluxes across isolated cornea of *Rana catesbeiana* before and during the action of prostaglandin E_1 at 10^{-5} M

J_{AT} (μEq hr^{-1} cm^{-2})		J_{TA} (μEq hr^{-1} cm^{-2})	
Control	PGE_1	Control	PGE_1
1.140 ± 0.059 n = 10	1.866 ± 0.056	0.160 ± 0.035 n = 6	0.284 ± 0.037
J_{Net} (μEq hr^{-1} cm^{-2})		J_{SCC} (μEq hr^{-1} cm^{-2})	
Control	PGE_1	Control	PGE_1
0.980 n = 19	1.582	1.020 ± 0.025 n = 19	1.610 ± 0.040

Means ± SE. *AT* = aqueous to tears, *TA* = tear to aqueous, SCC = short-circuit current, n = number of experiments. The fluxes were measured with ^{36}Cl in nonpaired groups of corneas. J_{SCC} was calculated from the average short-circuit current of all 19 controls and treated corneas.

Regardless of the order of administration, the combined effect of PGE_1 and Db cAMP was not significantly different from that due to Db cAMP alone (Table 3).

Chloride Fluxes

The undirectional fluxes of chloride were measured with ^{36}Cl in corneas before and during stimulation of the short-circuit current and potential difference with prostaglandins. The flux in each direction was measured in two separate groups of corneas. The results, shown in Table 4, indicate an increase in the unidirectional flux of chloride, in the direction of the transport, and a smaller increase in the passive flux from tear to aqueous side. The net increase in chloride flux is proportional to the increase in short-circuit current, and indicates that most of the effect of PGE_1 consists of a stimulation of chloride transport. The increase in the passive flux reflects an actual modification of the permeability which is consistent with other drugs that act through the cyclic AMP system such as epinephrine and theophylline (Chalfie *et al.*, 1972; Klyce *et al.*, 1973).

Action on Light Transmission

Frog corneas were examined for changes in light transmission while short-circuited in the plastic cells. The system has been described in detail by Zadunaisky and Lande (1971). In essence it consists of sending a beam

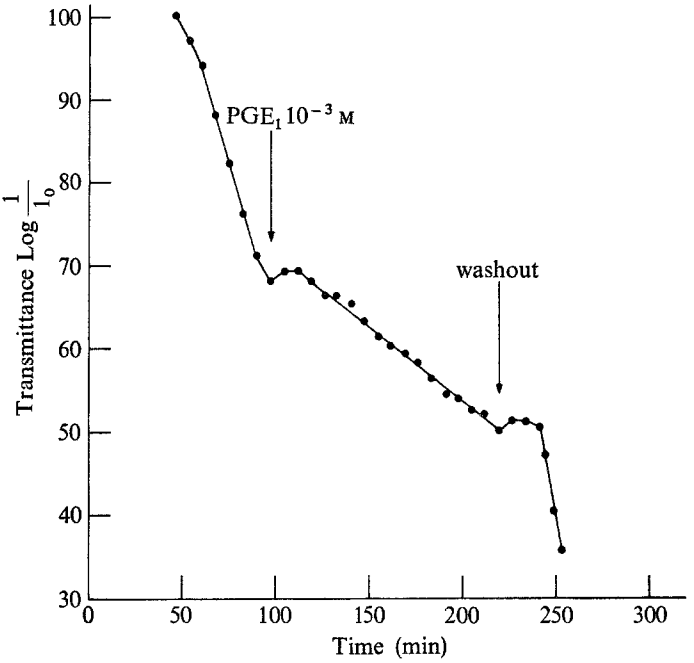

Fig. 7. Effect of PGE_1 on the transmittance of light through the cornea of the frog. The initial slope corresponds to the spontaneous reduction in light transmitted through a cornea mounted in a Lucite chamber, due to gain of water and scatter of light by the stroma. At the first arrow, PGE_1 produces a clear-cut reduction in the slope of the curve, and recovery is observed after washout with fresh Ringer's solution. Table 5 was composed with data for the slopes of control and treated periods of experiments similar to the one presented in this figure

of light at 5250 Å onto the epithelium and recording the light transmitted through the cornea with a photocell, placed on the aqueous side of the Lucite chamber. Fig. 7 shows the decline in light transmission by a frog cornea maintained in this condition and the change in slope produced by the administration of PGE_1. It can be observed that the loss of transmission changes drastically when prostaglandins are administered and at the time when the chloride transport is stimulated. This effect was observed in both corneas maintained at open circuit or short circuited. Table 5 gives the actual values of percentage change in light transmission produced by prostaglandins. At open circuit, there is a sevenfold reduction in the rate at which corneas swell and reduce their transmittance when treated with prostaglandins. Under short-circuited conditions, the effect is smaller, but 3 times as slowly with prostaglandins as in the controls. This indicates that the activation of the chloride pump of the epithelium induces a water move-

Table 5. Values of the slope of the transmittance of light at 5250 Å through isolated frog corneas (open and short-circuited) before and after treatment with prostaglandin E_1 in the endothelial side

Exp. no.	Control slope Δ-$\log_{10} T/t$ (% hr^{-1})	Treated with PGE_1 Δ-$\log_{10} T/t$ (% hr^{-1})	Ratio of slopes α
		Open circuit	
1	15.0	2.5	6.0
2	40.0	10.0	4.0
3	47.0	5.0	9.4
4	36.0	20.0	1.8
5	27.0	10.5	2.5
6	17.3	14.6	1.2
7	24.0	6.0	4.0
8	40.0	25.0	1.6
3′	24.0	12.0	2.0
4′	25.0	1.0	25.0
5′	14.0	0.5	28.0
6′	27.0	3.0	9.0
7′	23.3	5.3	4.4
Mean ± SE	27.6 ± 2.8	7.5 ± 2.07	7.6 ± 2.4
		Short-circuited	
1	32.0	4.0	8.0
2	24.0	14.6	1.6
3	9.3	8.3	1.1
4	31.0	5.0	6.2
5	17.0	14.6	1.1
6	7.0	3.0	2.3
7	14.0	9.5	1.5
8	9.3	0.6	15.5
9	12.0	6.0	2.0
2′	26.0	5.0	5.2
4′	18.0	10.0	1.8
5′	8.0	7.0	1.1
7′	9.2	8.0	1.1
9′	26.0	10.0	2.6
Mean ± SE	17.3 ± 2.37	7.5 ± 1.1	3.6 ± 1.1

The slope of the decrease of the extinction E, expressed as $-\log_{10} T$, where T is the transmittance is here expressed as the percent change per hour. The ratio α between the slopes (before and after treatment) gives the fractional reduction in E produced by PGE_1. For instance, at open circuit the corneas lost transparency about 7 times slower when PGE_1 was present in the bathing solution. The difference between controls and treated was statistically significant at a level of $p < 0.001$. Between controls, the difference between open circuit and short circuit was also statistically significant at $p < 0.01$. Numbers with prime (′) notation correspond to repeats of the effect of PGE_1 after recovery from the original dose in the same experiment.

ment out of the stroma that counteracts the leaks occurring during the experiment. This in turn reduces the amount of scatter occurring in the stroma. The transmittance of control corneas at open circuit is reduced more rapidly than in those which are short-circuited. The reason for this difference most probably resides in the fact that during short circuit the net transport of chloride is significantly greater than at open circuit.

Discussion

The addition of prostaglandins to the inside bathing solution induces an increase in the SCC of the isolated frog cornea. The effects are of a similar degree for the three prostaglandins analyzed here (PGE_1, PGE_2, and $PGF_{2\alpha}$) with maximal increases between 50 and 55%. The main difference resides in the dose necessary to produce a maximal response, which occurred at 1×10^{-5} M for PGE_1, 1×10^{-6} M for PGE_2, and 2×10^{-5} M for $PGF_{2\alpha}$. The potential difference maintained by the corneas also increased, but not proportionally to the increase in current, with a subsequent drop in the electrical resistance, between 0 and 30% of the original values.

The stimulation of the electrical parameters is a consequence of the proportional increase in the active transport of chloride across the corneas. This is substantiated by the requirement of chloride ions in the solutions bathing the tissue in order to obtain the prostaglandin response, and by the more direct evidence that the net chloride flux increases correspondingly with the SCC. The increase in net chloride flux was due to an elevation of the unidirectional chloride flux from aqueous to tear side, in the direction of the transport. A smaller increase in the passive chloride flux most probably indicates the effect of prostaglandins, or more accurately, of cyclic AMP on membrane permeability.

The action of prostaglandins was effectively blocked by PPP and DPP, which are known to have this inhibitory action on other systems. In the eye, Beitch and Eakins (1969) showed that the increase in intraocular pressure caused by prostaglandins was blocked by PPP. Eakins, Karim and Miller (1970) also demonstrated that PPP blocks the smooth-muscle-contracting effect of prostaglandins in isolated jird colon, rabbit jejunum and rabbit uterus. More recently, PPP has been shown to block the inhibitory action of prostaglandins on water flow stimulated by vasopressin in the toad bladder (Ozer & Sharp, 1972). Although prostaglandins inhibit the increased flow produced by vasopressin, they stimulate sodium transport across the frog skin (Fassina, Carpenedo & Santi, 1969) and the toad bladder (Orloff & Handler, 1965).

Polyphloretin phosphate (PPP) is a polyanionic polyester of phloretin and phosphoric acid which has been shown to possess antihyaluronidase activity (Diczfalusy *et al.*, 1953) in addition to its ability to antagonize various actions of prostaglandions. These two functions have been separated by Eakins (1971) and were shown to reside in a high-molecular-weight fraction and a low-molecular-weight fraction, respectively. The antagonism to prostaglandin is more predictable and reproducible with DPP than with PPP.

Both PPP and DPP are apparently inactivated if kept in contact with the frog cornea preparations for more than a few minutes. It was our experience that these substances had to be added 1 or 2 min before the prostaglandins in order to obtain inhibition. If they were added 15 min before the prostaglandins, no inhibitory effect was observed. This absence of effect could be due to inactivation by the tissue, though this matter was not investigated further.

The mechanism of action of PPP and DPP at the cellular level is not completely known; therefore, it was explored by testing their effects on the response of the frog cornea to epinephrine. If the inhibition by PPP and DPP consisted of a blocking effect at the level of a common receptor for both prostaglandins and epinephrine on the cell membrane, then they would block the response to epinephrine. The results tend to indicate that this is not the case; PPP and DPP are blocking either another specific receptor for prostaglandin or an allosteric region on the adenyl cyclase moiety of the cell membranes of the frog corneal epithelium.

In spite of the selectivity in the action of DPP, the mechanism of action of prostaglandins appears to be associated with epinephrine in the frog cornea. There was no response to prostaglandins after a maximal response to epinephrine; and in the reverse situation, further stimulation after a response to prostaglandin could be elicited with epinephrine. On the other hand, if the prostaglandin was administered when the response to epinephrine was fading away, as in Fig. 5, a small response was indeed obtained. This qualitative observation is in agreement with the theory of receptor sites, proposed also for substances that utilize cyclic AMP as their "second messenger" (Robison *et al.*, 1971). The actions, then, of prostaglandins on the chloride flux of the frog cornea seem to be mediated by the same mechanism as the effects of epinephrine, but they do not have a common inhibitor in DPP.

Furthermore, prostaglandins appear to act through cyclic AMP in the cornea, as in many other systems (*see* Ramwell & Rabinowitz, 1971). The experiments with Db cAMP give results essentially similar to those with

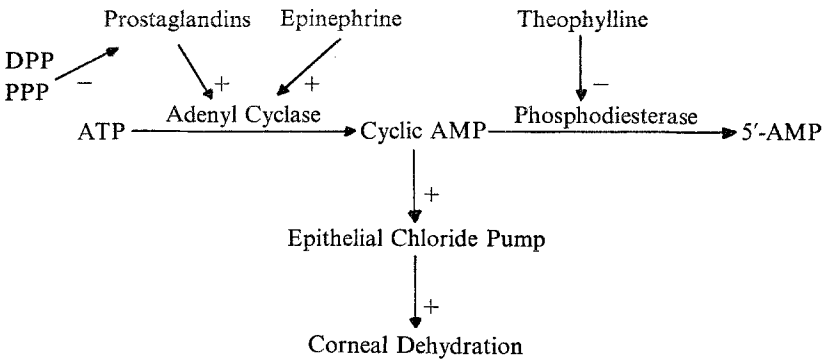

Fig. 8. Simple diagram of the relationship of PGE_1, epinephrine, etc., to the chloride pump of the frog corneal epithelium

epinephrine; that is, no significant response to prostaglandin at the height of the response to Db cAMP and a doubling of the SCC with Db cAMP after activation with prostaglandin. However, Db cyclic AMP gives a slower and more sustained response than epinephrine. This is probably due to the rate of penetration of Db cyclic AMP (Robison *et al.*, 1971).

The interaction of theophylline with prostaglandins differs from the previous ones. With theophylline, the effects were additive, regardless of the order of addition of the two drugs. Therefore, in contrast to epinephrine and Db cAMP, there was a response to PGE_1 after maximal effect of theophylline. This suggests, then, that a further increase in cyclic AMP content of the tissue is elicited by prostaglandins after stimulation with theophylline, and suggests that no direct interaction of prostaglandins with phosphodiesterase occurs in the frog cornea.

A general and simplified view of the interactions of PGE_1, epinephrine, cyclic AMP, and theophylline on the frog cornea is given in Fig. 8.

The actual role of the release of native prostaglandins in the eye is not completely clear. Prostaglandins appear in the aqueous humor in acute, spontaneous, or experimental inflammations. There is also an increase in prostaglandin in the aqueous humor after trauma, which is accompanied by constriction of the pupil, an elevated protein level and an increase in the intraocular pressure (*see* Eakins, 1973).

A possible normal function of the prostaglandins in the cornea is the regulation of the ionic pumps and water movements that control hydration and transparency. The experimental evidence presented here shows that prostaglandin increases the light transmitted through opaque corneas and certainly points in the direction of a role of the epithelium in the control

of hydration and transparency of the corneal stroma. The partially opaque, swollen corneas are sensitive to prostaglandin and become more clear when the potential or the current is raised by this drug. The increase in chloride transport, then, can elicit a further movement of water out into the tears that will clear the partially opaque corneas. In fact, this type of increase in light transmission due to stimulation of chloride transport of the epithelium was demonstrated before in other experimental conditions (Zadunaisky & Lande, 1971).

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