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Cl Secretagogues Reduce Basolateral K Permeability in the Rabbit Corneal Epithelium

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Abstract. The stromal-to-tear transport of Cl by the rabbit corneal epithelium is increased by pharmacological effectors (secretagogues) that raise cAMP. It is well established that such secretagogues increase the apical membrane permeability to Cl and thus facilitate the efflux of the anion. However, we and others have found that cAMP-elevating agents frequently decrease the transepithelial potential difference across the rabbit cornea. The mechanism underlying this latter phenomenon had not been characterized. In this report, transepithelial and microelectrode studies were combined with measurements of unidirectional fluxes of ^{36}Cl, ^{22}Na and ^{86}Rb to show that secretagogues known to act via cAMP also decrease the K permeability of the basolateral membrane, which by cellular depolarization would decrease apical Cl secretion. This effect was increasingly pronounced as a function of concentration when agents (e.g., epinephrine, isoproterenol) were applied to the apical side of the preparations. The addition of these agonists to the basolateral bathing solution, or of forskolin to the apical side, solely elicited inhibitions of basolateral K permeability. It seems that apical Cl and basolateral K conductances are independently and inversely regulated by cAMP. The opposite effects that cAMP could have on fluid secretion and epithelial thickness, by increasing apical Cl permeability but decreasing basolateral K permeability, may serve as a mechanism to maintain epithelial thickness within a narrow range.

Key words: Short-circuit current — Microelectrode study — Ion transport — Adrenergic compounds — Unidirectional Cl, Na and Rb fluxes

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Introduction

The corneal epithelium serves as a model for Cl-secreting epithelia where the basolateral membrane absorbs Cl into the cell via a Na:K:2Cl cotransporter and releases the anion into the apical milieu via Cl channels [5, 22, 33] possibly including the CFTR [1, 18].

Such secretion is stimulated by so-called Cl secretagogues that include various cAMP-elevating agents and Ca ionophores [8, 9, 12, 19, 25, 28, 30], which primarily increase the Cl permeability of the apical membrane. Simultaneously to the action on apical Cl permeability, secretagogues also increase the basolateral K permeability of the frog corneal epithelium (FCE), an effect that potentiates, by cellular hyperpolarization, the net basolateral-to-apical Cl flux [40].

In the FCE a substantial ($\approx$15 $\mu A/cm^2$) short-circuit current (I_{sc}) is almost exclusively generated by the Cl transport, with a residual 5–10% generated by an apical-to-basolateral Na transport [7].

In contrast, the I_{sc} across the rabbit corneal epithelium (RCE) is smaller ($\approx$5 $\mu A/cm^2$) and generated in roughly equal parts by Cl secretion and Na absorption [20, 23, 38]. Cl secretagogues increase the I_{sc} of RCE by increasing apical Cl permeability [19, 25]. However, in many cases this stimulation in I_{sc} is accompanied by a decrease in the transepithelial potential difference (PD_t) [8, 13, 20, 21, 23–25]. No inhibition of the I_{sc} was reported in these previous publications in response to Cl secretagogues. We have recently found that frequently the cAMP-elevating secretagogues cause an inhibition not only of the PD_t but also of the I_{sc}. In comparing the effects of forskolin on frog and rabbit corneal epithelia, we noticed that in the RCE, it produced only a small and brief stimulation of the I_{sc}, followed by a moderate inhibition. Thus we decided to investigate the causes of this dual effect.

There are reports in other tissues that elevated cellular cAMP or Ca reduce basolateral K permeability [2, 14, 15, 27, 29] rather than increase it as seen in the FCE [40]. We thought that a reduction of the basolateral K permeability, which depolarizes the cell, would decrease apical Cl exit and thereby explain the inhibition of the I_{sc} in the RCE. In this work we present experiments designed to determine the effects of various Cl secretagogues on the basolateral K permeability of the RCE.

Materials and Methods

Adult albino and/or Dutch-belted rabbits of either sex weighing 2–3.6 kg were killed by CO_2 asphyxiation. The tissues were mounted in either of two Ussing-type chambers, one for short-circuit current and unidirectional radioisotope flux measurements and a second for intracellular recordings. With either approach, the hemichambers included the necessary arrangements for electrical determinations and vigorous stirring.

Transepithelial Electrical Measurements

Corneas were mounted as a membrane between the hemichambers as previously described [6–10], exposing a 0.5-cm^2 area to the bathing solutions. The tear-side compartment contained 5 ml Tyrode's solution and the stromal-side one had 6 ml, thus maintaining the natural curvature of the cornea by a minimal hydrostatic pressure difference.

The PD_t-sensing bridges were kept close to each respective surface and were connected through Ag/Ag Cl electrodes to an external automatic voltage clamp system. The transcorneal potential difference was short-circuited, with the current needed to maintain 0 mV across the tissue (the I_{sc}) being continuously recorded. Transmural electrical resistance (R_t) was determined by measuring the amount of current necessary to offset the short-circuit condition by 5 mV for a few seconds. The Tyrode's solution (composition below) bathing each corneal surface was continuously bubbled with a humidified 5% CO_2-95% air mixture and maintained at 37°C by heating coils inserted within each bath.

Intracellular Electrical Measurements

Corneas were mounted in a vertically oriented Ussing-type chamber designed for microelectrode impalements [6, 8]. A thin but rigid, stainless steel dome-shaped grid was placed on the lower hemichamber to support the stroma with the epithelium facing upward. The top end of the upper hemichamber had an aperture for the microelectrode, enabling penetration of the epithelium from the apical side. Tyrode's solution was re-circulated on each side of the cornea by gas bubbling in funnels connected to the chamber and positioned so that 10-cm hydrostatic pressure was applied to the cornea to keep it fixed against the dome. The volume of the bathing solution on each side of the cornea was 15 ml. Intracellular recordings of PD_i were obtained with microelectrodes prepared from glass capillaries (1 mm OD and 0.58 mm ID) that had been pulled with a vertical pipette puller. The microelectrodes were backfilled with filtered 1 M KCl. Their tip resistances were between 15 and 40 MΩ, and their tip potentials never exceeded 5 mV. A tip diameter of 0.2 μm was established previously [6, 8]. Cellular impalements were done perpendicular to the corneal surface, inside a grounded copper-shielded Faraday cage, with a Stoelting MM-5M motorized micro-manipulator under visual control. The signal was amplified with a WPI M707 amplifier, displayed on a Keithley digital electrometer, and recorded on a chart recorder.

The corneas were kept short-circuited during the impalement; thus the PD was the same across the basolateral and apical surfaces. The criteria for acceptance of impalements were similar to those described previously [6, 8]. Ra/Rb was determined from the deflections in PD_i across the basolateral side compared with the imposed PD pulse across the tissue.

Unidirectional Na, Cl and Rb fluxes

Unidirectional fluxes of Na, Cl or Rb were measured by adding either 5 μCi ^{22}Na, 5 μCi ^{36}Cl or 30 μCi ^{86}Rb, respectively, to one chamber compartment and taking periodic samples from the opposite compartment. The specific activity of the labeled solution remained constant through the experiment, and the activity of the opposite solution was always ~0.001 of the labeled side. Two-milliliter samples were taken every 15 min from the unlabeled side, the volume of which was kept constant by immediate addition of fresh medium. Twentyfive micro-liter samples were taken from the labeled side and diluted up to 2 ml with bathing medium in order to determine the specific activity of the labeled solution. The samples were counted with a Packard Cobra II Gamma Counter or a Wallac Scintillation Counter.

Solutions

The basic physiological medium used to bathe both sides of the cornea was a modified Tyrode's solution that contained (in mM): 1.8 $CaCl_2$, 1.2 $MgCl_2$, 4.5 KCl, 103 NaCl, 30 $NaHCO_3$, 1 NaH_2PO_4, 5.6 glucose and 10 sucrose and had a measured osmolality of 290 ± 5 mOsmole/kg H_2O.

For experiments requiring a transepithelial K gradient, the solution bathing the apical surface of the cornea had a cell-like [K] and contained (in mM): 1.8 $CaCl_2$, 1.2 $MgCl_2$, 9 KCl, 14 Na gluconate, 61 K gluconate, 30 $KHCO_3$, 1 NaH_2PO_4, 5.6 glucose and 56.3 sucrose. In Cl-free experiments, the anion was replaced by equimolar amounts of sulfate and gluconate. When needed, K salts were completely replaced by Rb salts in the solutions described.

Chemicals

All pharmalogical agents were purchased from Sigma (St. Louis, MO), except for amphotericin B (APOTHECON, Princeton, NJ). $^{22}NaCl$ and $H^{36}Cl$ were purchased from Perkin Elmer Life Sciences, Inc. (Boston, MA). $^{86}RbCl$ was obtained from Nycomed Amersham (Arlington Heights, IL).

Results

Effects of Cl Secretagogues on RCE Bathed in Tyrode's solutions

Previous reports in the literature indicate that epinephrine stimulates the I_{sc} in the RCE [21, 23] but no inhibition had been reported. Although we also observed such stimulation when epinephrine was added to the apical side solution [10], an inhibition was obtained when added to the basolateral side in an increasing dose. The I_{sc} of the RCE responds in a

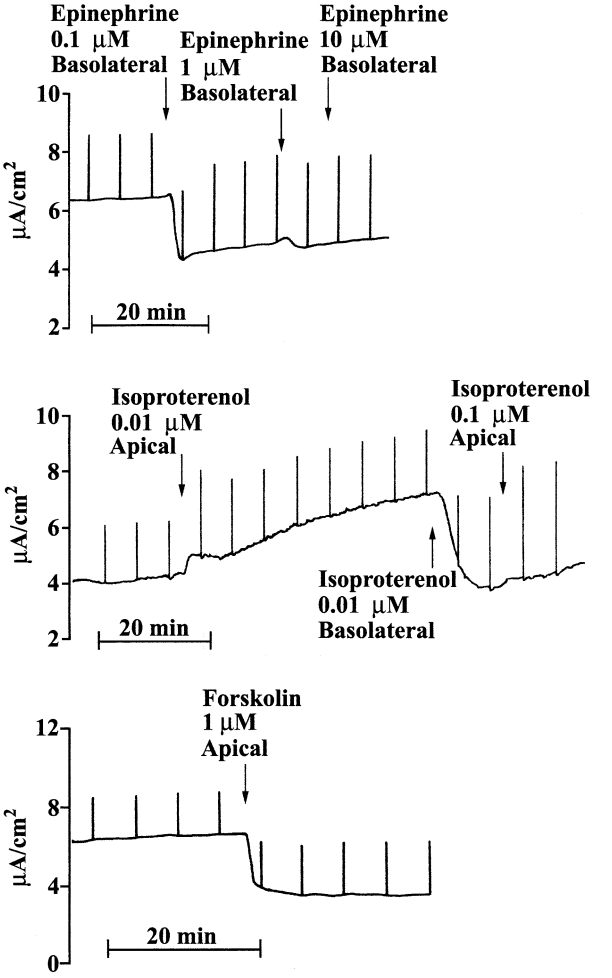

Fig. 1. Representative experiments illustrating effects of various Cl secretagogues on the rabbit corneal transepithelial I_{sc}. Spikes are deflections in I_{sc} produced by a *PD* signal to determine transepithelial resistance. *Top panel:* epinephrine addition to the basolateral side at three increasing concentrations. *Middle panel:* sidedness of effect of isoproterenol at 10^{-8} M. *Bottom panel:* single application of forskolin to the apical bathing solution.

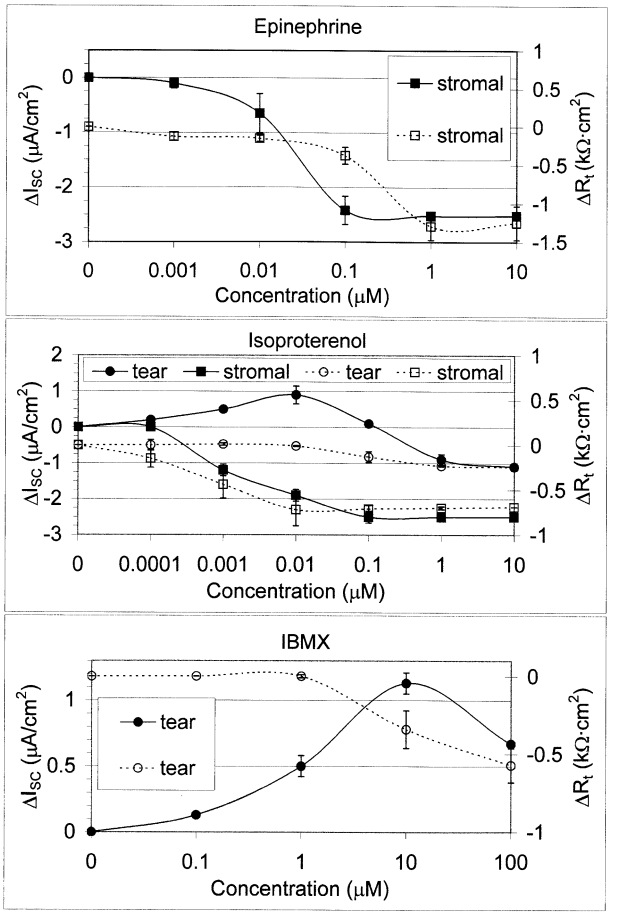

Fig. 2. Dose response of rabbit corneal transepithelial electrical parameters to sequential additions of various secretagogues. Each point represents the succeeding change ($n \geq 3$) in short-circuit current (left axis; solid trace and filled symbols), transepithelial resistance (right axis; dashed trace and empty symbols) at the doses indicated. The terms "stromal" and "tear" denote changes in the electrical parameters due to the exclusive addition of an agent to the stromal and tear-side baths respectively. Control I_{sc} and R_t were for epinephrine: 7.50 ± 0.22 μA/cm² and 2.25 ± 0/15 KΩ cm² ($n=5$); isoproterenol tear: 5.41 ± 0.58 μA/cm² and 2.89 ± 0.26 KΩ cm² ($n = 8$); isoproterenol stromal: 6.25 ± 0.38 μA/cm² and 3.17 ± 0.21 KΩ cm² ($n = 6$); and IBMX: 3.50 ± 0.20 μA/cm² and 3.33 ± 0.34 KΩ cm² ($n = 4$) respectively.

similar fashion to the β adrenergic agonist isoproterenol. Forskolin always inhibited the I_{sc}. Typical results for these agents are shown in Fig. 1.

Epinephrine at 10^{-7} M on the basolateral side produced an immediate inhibition of the I_{sc}. Larger doses did not significantly alter the already lowered I_{sc}. Isoproterenol on the apical side produced a prompt stimulation of the I_{sc} that continued to increase for about 30 min. At that point, the same dose on the basolateral side reduced the I_{sc}, which was not modified by the subsequent dose of 10^{-7} M to the apical-side solution. Forskolin, from the apical side, also reduced the I_{sc} significantly.

In Fig. 2 we have compiled the results in a dose-dependent format for basolateral epinephrine, apical IBMX and isoproterenol. With epinephrine, an inhibitory effect on the I_{sc} becomes evident at 10^{-8} M and is accentuated at higher concentrations. The electrical resistance also declines at high concentrations. This unexpected result may be associated with a change in paracellular permeability, which will have no influence on the I_{sc}. Since epinephrine is both a β and α agonist, we tested a specific β agonist, isoproterenol. The dependence on concentration and side of addition is evident with this adrenergic agonist. Increasing concentrations from the apical side produced increasing stimulation of the I_{sc} followed by inhibitions at 10^{-6} M and higher concentrations. There were only inhibitions of the I_{sc} from the basolateral side. As with epinephrine, R_t only declined with the high concentrations. IBMX was only tested

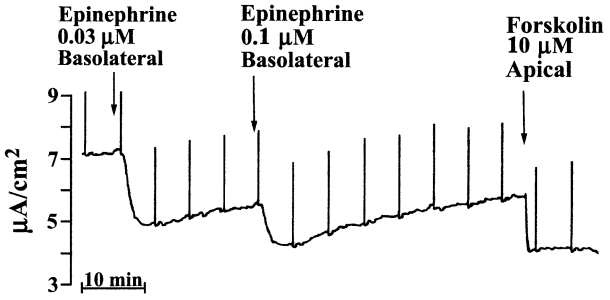

Fig. 3. Sequential effects of epinephrine and forskolin on transepithelial I_{sc} of rabbit cornea.

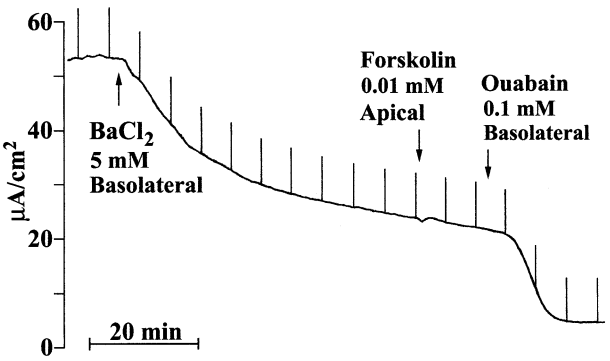

Fig. 4. Effects of $BaCl_2$, forskolin and ouabain on the transepithelial I_{sc} of a rabbit cornea bathed bilaterally with control, Cl-rich Tyrode's and pretreated with amphotericin B.

on the apical side and it stimulated the I_{sc} moderately at doses between 10^{-7} and 10^{-4} M.

It seems that relatively low concentrations of the secretagogues added to the apical side stimulate the I_{sc}, whereas higher concentrations or basolateral addition inhibit the I_{sc}. It is not clear why the secretagogues have this dual effect that is apparently associated with the cellular concentration of cAMP. Forskolin always inhibited the I_{sc}, even after a maximum inhibitory effect was obtained with one of the other secretagogues (Fig. 3). Since the I_{sc} ultimately depends on the activity of the Na:K pump, it was possible that the inhibitory effect on the Cl-originated I_{sc} was secondary to an inhibition of the Na:K ATPase, which fuels the Na:K:2Cl cotransporter. To test this possibility, the apical membrane was permeabilized with amphotericin B, a maneuver that elicits a large Na minus K-originated I_{sc} produced by the basolateraly located Na:K pump plus a recirculation of K across the basolateral membrane [7]. As seen in Fig. 4, after the K current is eliminated by Ba, forskolin does not have an additional effect. However, ouabain immediately inhibited the remaining I_{sc}, indicating that forskolin does not inhibit the Na:K pump. The larger inhibitory effect by Ba than by ouabain suggests a large K recirculation across the basolateral membrane.

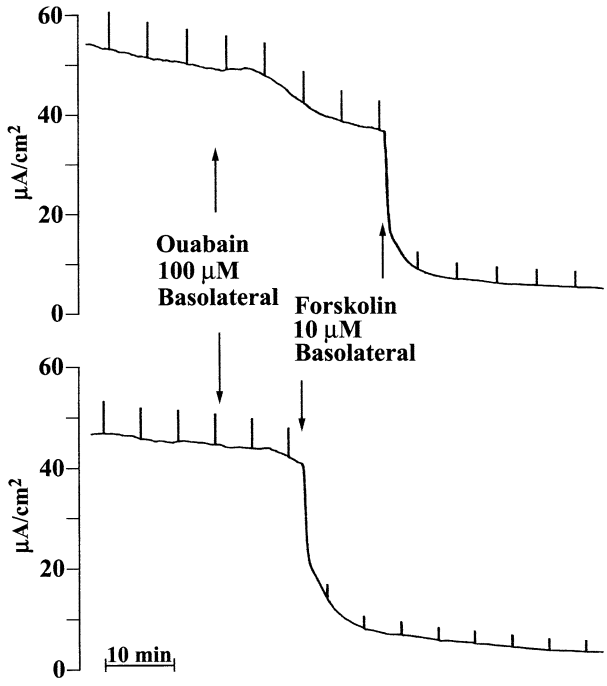

Fig. 5. Effect of forskolin on the K-originated I_{sc} produced by an apical-to-basolateral K gradient and amphotericin B pretreatment. Top and bottom panels, represent Cl-rich and -free bathing solutions respectively.

Effects of Secretagogues on I_{sc} and Ionic Fluxes across RCE Exposed to an Apical-to-Basolateral K Gradient

Having ruled out that the Na:K pump is inhibited, three alternatives for explaining the observed inhibitory effect of forskolin can be advanced: 1, that elevated cellular cAMP concentration reduces apical Cl permeability; 2, that it inhibits the basolateral Cl cotransporter; or 3, that it reduces basolateral K permeability, thus reducing apical Cl exit by depolarization when the apical membrane is intact.

To test for these possibilities, the effects of forskolin were determined in tissues in which the apical membrane was exposed to a high [K] solution and permeabilized by amphotericin B. In this condition, the apical Cl permeability is irrelevant since Cl can easily exit the apical membrane across the amphotericin B-formed channels [4, 16] and most of the observed I_{sc} is not generated by the Na:K pump, as determined by the lack or minimal effect of ouabain as previously show [36, 40]. This was confirmed in several experiments in this study. Most likely, this I_{sc} is the sum of an apical-to-basolateral K current (driven by its gradient) plus a basolateral-to-apical Cl current, produced by the Cl cotransporter. As shown in Fig. 5, the amphotericin B-stimulated I_{sc} is only slightly affected by ouabain but drastically reduced by forskolin. Such inhibition is also seen when the K-gradient experiments are conducted in Cl-free

solutions, a condition in which the apical Cl channels and the Na:K:2Cl cotransporter are not involved in the generation of the I_{sc} (Fig. 5, lower panel). Table 1 summarizes the inhibitory effect of forskolin on the I_{sc}. Notice that in this case, in which the current is controlled by the basolateral K channels, the resistance increases substantially, indicating channel closure. Effects by epinephrine and other cAMP-elevating agents were merely surveyed and statistics are presented when the number of trials was 3 or more. As a group, however, all agents reduced the I_{sc} and increased the basolateral resistance. The effects were observed in both Cl-rich and Cl-free solutions. Particularly impressive were the effects of epinephrine at 10^{-7} and 10^{-6} M and of forskolin. Rolipram, a phosphodiesterase inhibitor, as well as terbutaline and dobutamine, selective β agonists, had potent inhibitory effects on the I_{sc}. These results clearly point to the basolateral K channels as the site of action by forskolin and the other Cl secretagogues for the decrease in I_{sc}. Another indication of an effect on a basolateral channel is the consistent increase in R_t.

To further exclude an effect on Cl and Na fluxes, unidirectional fluxes of these ions, as well as of ^{86}Rb, were also measured in the presence of a tear-to-stromal K gradient and the results are summarized in Table 2. The top panel shows the unidirectional Cl fluxes in both directions. In control conditions the stroma-to-tear side (basolateral-to-apical) is larger than the opposite side because of the Na:K:2Cl cotransporter. The small tear-to-stroma (apical-to-basolateral) flux may simply represent paracellular movement. Amphotericin B produces a discrete increase in both fluxes with a net flux (≈6.8 μA/cm^2), which is far less than I_{sc}, indicating that the I_{sc} is carried by another ion(s). Forskolin produces a substantial (at least 20 μA/cm^2) reduction in the I_{sc}, but only a small increase in the Cl fluxes. With this experimental protocol, Cl can only account for a small fraction of the I_{sc}, and more importantly, Cl fluxes are not inhibited by forskolin. Bumetanide only has a small inhibitory effect on the stroma-to-tear flux, consistent with an inhibition of the Na:K:2Cl cotransporter.

The center panel shows the unidirectional Na fluxes. As with Cl, the fluxes were small and a large part may represent paracellular movement. Amphotericin B produced the typical large increase in I_{sc}, but only a modest increase in the tear-to-stroma flux, possibly due to a stimulation of the Na:K pump after the apical barrier was permeabilized. The net Na flux of about 4 μA/cm^2 cannot account for the large 50 μA/cm^2. Forskolin and bumetanide had insignificant effects on the Na fluxes, even though forskolin produced its typically large inhibition of the I_{sc}.

The bottom panel shows the same experimental protocol on ^{86}Rb unidirectional fluxes used as a label for K. In control condition the fluxes were very small, probably limited to movement across the paracellular pathway, and roughly proportional to the concentrations of K in the bathing solutions. Amphotericin B produced the typical effect on the I_{sc}, but a lower than anticipated increase in the ^{86}Rb fluxes, although a net flux (14.1–7.6) is evidenced. Forskolin inhibited the I_{sc} and eliminated the net Rb flux. Bumetanide was without an effect. The only explanation for the results in Table 2 is that forskolin inhibits the basolateral K permeability but ^{86}Rb is not an identical replacement for K.

Effects of Replacement of Rb for K on the I_{sc}

Using a protocol with the same solutions as in the previous section (“cell like”, high [K] in the apical solution), the 100 mM [K] in the apical solution was replaced, after amphotericin B stimulation of the I_{sc}, by increasing concentrations of Rb. Figure 6 shows results from a typical experiment. Replacing 2/3 of the volume of the apical bathing solution with one containing 100 mM RbCl (and the other components remaining constant), the K concentration was reduced to 33.3 mM and the Rb concentration set at 66.7 mM. The I_{sc} immediately decreased by 26%. Further increase of the Rb concentration to a Rb/K ratio of 87.8/22.2 produced a further decrease of the I_{sc} to 64% of its initial value. Concomitant with the reduction in I_{sc}, Rb also increased R_t by about 13%. Removal of Rb by total solution replacement with the original KCl concentration restored the I_{sc} to almost its initial control value. Clearly, the basolateral K channels are less permeable to Rb than to K, which explains the discrepancy between the net ^{86}Rb fluxes and the I_{sc}.

Effects of Epinephrine on the Intracellular PD and Resistance Ratio

We have previously shown in the FCE that forskolin depolarizes the intracellular *PD* (PD_i) and reduces the resistance ratio (Ra/Rb) by 50% [8]. In the RCE the depolarization was much larger and the Ra/Rb was reduced by 80% [8]. No clear interpretation of the difference between frog and rabbit was then given. Now we understand that the difference in the changes in Ra/Rb between the two species is due to the fact that in the RCE the apical Cl channel opens while the basolateral K channel closes with forskolin application. We confirmed this interpretation with 3 experiments in which RCE were impaled with microelectrodes. One of these experiments is shown in Fig. 7. Epinephrine at 10^{-5} M on the stromal side reduced the I_{sc} by a few μA, PD_i by 30 mV, and Ra/Rb by a factor of 3. Such combined changes were never observed in the FCE, in which the Cl secretagogue increases both the apical Cl

Table 1. Effects of the Cl secretagogues on the I_{sc} and R_t of isolated rabbit corneal epithelia pre-treated with amphotericin B in the presence of an apical-to-basolateral K gradient

Secretagogue		Cl-rich solutions							Cl-free solutions						
		n	Baseline		Experimental		% change		*n*	Baseline		Experimental		% of change	
			I_{sc}	R_t	I_{sc}	R_t	I_{sc}	R_t		I_{sc}	R_t	I_{sc}	R_t	I_{sc}	R_t
Forskolin (apical)	10^{-5} M	28	42.8 ± 2.4	0.99 ± 0.13	8.7 ± 0.3	1.93 ± 0.08	−79.7[b]	94.9[b]	5	30.4 ± 6.8	1.26 ± 0.17	7.0 ± 0.7	2.51 ± 0.28	−77.0[b]	99.2[b]
Epinephrine (apical)	10^{-7} M	3	63.0 ± 5.6	0.51 ± 0.39	62.7 ± 7.3	0.54 ± 0.57	−0.4	5.9	3	34.4 ± 5.2	1.13 ± 0.04	30.4 ± 0.3	1.20 ± 0.05	−11.6	6.2
Epinephrine (basolateral)	10^{-10} M	3	46.3 ± 8.1	0.78 ± 0.15	42.9 ± 7.9	0.88 ± 0.43	−7.4[b]	12.8	4	36.8 ± 7.9	1.11 ± 0.25	34.6 ± 6.9	1.19 ± 0.30	−6.0[c]	7.2
	10^{-9} M	3	40.2 ± 5.0	0.90 ± 0.42	32.0 ± 6.7	1.05 ± 0.47	−20.4[b]	16.7	4	34.6 ± 6.9	1.19 ±0.30	30.8 ± 5.2	1.26 ± 0.27	−11.0[c]	5.9
	10^{-8} M	3	45.6 ± 4.9	0.76 ± 0.32	31.5 ± 3.9	1.25 ± 0.27	−30.9[b]	64.5	4	30.8 ± 5.2	1.26 ± 0.27	28.2 ± 5.3	1.40 ± 0.32	−8.4[c]	11.1
	10^{-7} M	3	44.0 ± 6.7	0.85 ± 0.46	15.8 ± 4.8	1.56 ± 0.29	−64.1[b]	83.5	5	27.1 ± 4.7	1.30 ± 0.18	12.9 ± 2.6	1.81 ± 0.25	−52.4[c]	39.2
	10^{-6} M	3	44.3 ± 3.6	0.90 ± 0.32	8.1 ± 3.5	1.89 ± 0.31	−81.7[b]	110.0	4	16.5 ± 2.4	1.68 ± 0.22	8.5 ± 0.7	1.98 ± 0.26	−48.5[c]	17.9
Rolipram (apical)	10^{-5} M								2	21.7	1.44	8.8	1.84	−59.4	27.8
Terbutaline (bas.)[a]	10^{-5} M								3	15.6 ± 3.0	1.60 ± 0.08	4.9 ± 0.3	1.97 ± 0.11	−68.6[c]	23.1
Dobutamine (basolateral)	10^{-5} M								2	27.2	1.34	10.6	1.67	−61.0	24.6
followed by Terbutaline	10^{-5} M								2	10.6	1.67	5.2	1.79	−50.9[a]	7.2

Data are expressed as mean ± SE or as mean when $n < 3$.

[a]Dobutamine (a β_1 adrenergic agonist) did not exert a further effect when added after Terbutaline (a β_2 adrenergic agonist). However, Terbutaline after Dobutamine produces an additional 51% decrease in I_{sc}. [b,c]Change is significantly different from zero as paired data. [b]$P < 0.01$. [c]$P < 0.05$.

Table 2. Unidirectional fluxes of ^{36}Cl, ^{22}Na and ^{86}Rb across isolated rabbit comeal epithelia in the presence of an apical-to-basolateral K gradient

	Cl flux tear-to-stroma (n = 4)			Cl flux stroma-to-tear (n = 5)		
	Flux (μA)	I_{sc} (μA)	R_t (kΩ cm^2)	Flux (μA)	I_{sc} (μA)	R_t (kΩ cm^2)
Control	0.27 ± 0.02	3.00 ± 0.18	9.10 ± 0.77	5.36 ± 0.64	4.16 ± 0.63	5.20 ± 0.34
Amphotericin B	0.89 ± 0.17[a]	42.35 ± 3.15[a]	0.92 ± 0.06[a]	7.65 ± 1.11[b]	29.80 ± 9.76[b]	1.79 ± 0.63[a]
Forskolin	1.38 ± 0.13[a]	8,33 ± 0.59[a]	1.88 ± 0.13[a]	8.92 ± 0.64[b]	7.30 ± 1.18[b]	2.20 ± 0.37
Bumetanide	1.45 ± 0.12	7.80 ± 0.64[a]	1.91 ± 0.13	7.41 ± 0.39[a]	6.58 ± 1.18[a]	2.34 ± 0.50
	Na flux tear-to-stroma (n = 4)			Na flux stroma-to-fear (n = 4)		
Control	1.31 ± 0.16	2.83 ± 0.41	6.56 ± 0.80	3.04 ± 0.31	5.48 ± 0.97	5.03 ± 0.66
Amphotericin B	7.48 ± 0.37[a]	47.15 ± 4.17[a]	0.77 ± 0.06[a]	3.85 ± 0.66	51.58 ± 3.03[a]	0.67 ± 0.05[a]
Forskolin	7.18 ± 0.68	8.56 ± 0.9[a]	1.95 ± 0.11[a]	5.51 ± 1.03[b]	9.38 ± 0.31[a]	1.77 ± 0.15[a]
Bumetanide	5.17 ± 0.65[a]	6.91 ± 0.61[b]	2.15 ± 0.18	5.71 ± 1.45	8.38 ± 0.48[b]	1.70 ± 0.17
	K(Rb) flux tear-to-stroma (n = 5)			K(Rb) flux stroma-to-tear (n = 2)		
Control	1.75 ± 0.30	4.64 ± 1.18	7.60 ± 0.84	0.11	4.15	8.01
Amphotericin B	14.09 ± 1.61	45.10 ± 3.39	0.85 ± 0.08	7.64	52.90	0.73
Forskolin	7.73 ± 0.59	10.02 ± 0.43	1.68 ± 0.07	9.25	9.95	2.07
Bumetanide	8.32 ± 0.65	8.74 ± 0.35	1.74 ± 0.05	9.78	9.85	2.09

Data are expressed as mean ± SE.
[a] Significantly different from previous value as paired data. $P < 0.01$. [b] $P < 0.05$.

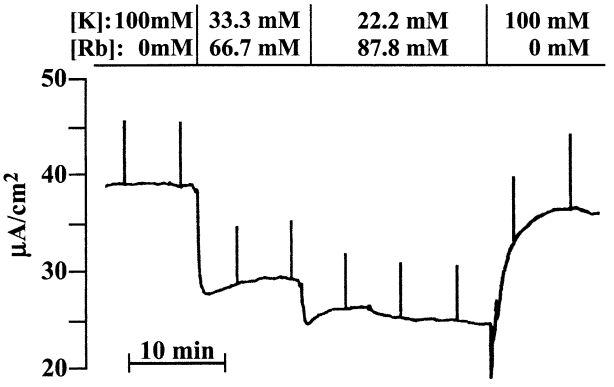

Fig. 6. Effects of K substitutions by Rb on the amphotericin B-induced apical-to-basolateral K current.

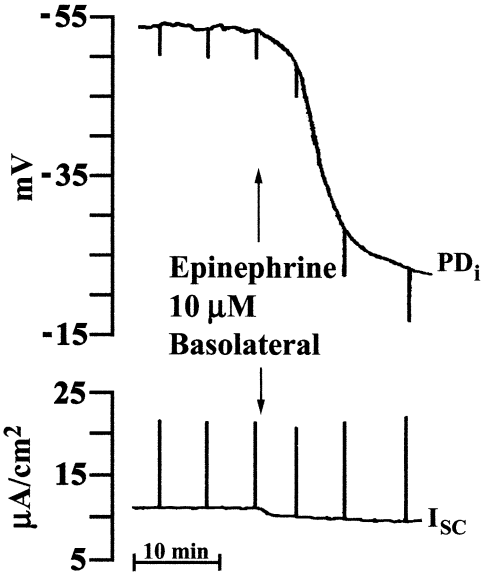

Fig. 7. Effect of epinephrine on intracellular PD (PD_i) and simultaneous I_{sc} and Ra/Rb measurements of a rabbit corneal epithelium bathed in Tyrode's solution. Deflections on PD_i trace represent voltage drop across the basolateral membrane with respect to a preimposed 13-mV pulse across the entire cornea. Deflections on I_{sc} trace represent transepithelial conductances in response to the pulse. From these parameters, values for Ra/Rb before and after epinephrine of 2.42 and 1.00 respectively can be calculated. Similarly, R_t was 1.30 KΩ and 1.00 KΩ, respectively.

and basolateral K permeabilities [40]. A reduction of I_{sc}, in combination with a depolarization of PD_i and a reduction in Ra/Rb can only occur if Rb increases. It should be noted, however, that following stimulation of PKC with an active phorbol ester analogue, also in the FCE epinephrine inhibited the I_{sc}, lowered Ra/Rb and depolarized the intracellular PD [31].

Discussion

Earlier studies on the effects of epinephrine in the RCE indicated that the adrenergic agonist as well as other cell cAMP-elevating agents stimulated the I_{sc} [13, 21, 23, 24]. No mention of I_{sc} inhibition is found in these reports. It was assumed that the increase in I_{sc} was due to a stimulation of the Cl current since the effect was absent in Cl-free solutions. In 1977, Kylce and Wong [25] formally reported, for the first time, that in some cases epinephrine decreased the PD_t of the RCE. This

effect was also seen but not addressed in virtually all publications in which secretagogues were used in the RCE [13, 20, 21, 23, 24]. In 1986, Candia et al. [8] described that forskolin produced only a transitory stimulation of the PD_t of the RCE followed by an inhibition. The decrease in Ra/Rb was also substantial (5-fold). A similar change in Ra/Rb was described previously with epinephrine and serotonin [25, 28]. Clearly, the rabbit corneal epithelium does not respond to Cl secretagogues consistently with an increase in the I_{sc} as in the case of FCE. Most of the studies reported the sensitivity to epinephrine from the apical side or both sides but rarely from the basolateral side exclusively, perhaps because a positive response could not be elicited from the basolateral side. Our present results in the RCE bathed in Cl-rich Tyrode's showed stimulation of the I_{sc} with only low concentrations of isoproterenol and IBMX from the apical side.

Additions of epinephrine and isoproterenol to the basolateral side always reduced the I_{sc}. This initial data already suggested that β-adrenergic receptors coupled to adenylate cyclase were present at the basolateral membrane, or that larger increases in cAMP could be obtained by basolateral than apical addition. Furthermore, these epinephrine and isoproterenol effects were blocked by the β antagonist propranolol.

This and the pattern of stimulation/inhibition of the I_{sc} suggested two targets for the second messenger that affected the I_{sc}. The first, the apical Cl channel(s), was first stimulated with the lower concentration from the apical side, and then a second element was sensitive to the higher concentrations of cAMP.

It is not possible to determine from the electrophysiological results if the dual effect is due to cAMP compartmentalization, proximity between the receptor and the effector, differences in sensitivity between the apical and basolateral receptor, or a combination of those factors. In any case, stimulation from the basolateral side seems to overwhelm the increase in apical Cl permeability with a consequent decrease in I_{sc}.

When the RCE was bathed with a high [K] solution on the tear side and the apical barrier was permeabilized with amphotericin B, the I_{sc} almost exclusively reflected an apical-to-basolateral K current across the basolateral membrane as shown before [40]. In this condition epinephrine, from either side, reduced this I_{sc}, but at a given concentration, the inhibition was much larger from the basolateral side. The presence of Cl in the solutions was irrelevant to the reduction of this K current. Forskolin inhibited this K-originated I_{sc} by about 79%, similar to the maximum inhibition produced by epinephrine. Rolipram also exerted a large reduction in I_{sc}. Two β-adrenergic agonists (one β_1 and another β_2) also reduced the I_{sc} by a significant 61–68%. The I_{sc} reductions were associated with an increase in R_t.

The most plausible interpretation is that cell cAMP-elevating agents reduce the K current across the basolateral membrane since the apical membrane has been permeabilized. However, Wolosin [39] suggested that the reduction in current could be due to closing of gap junctions between cell layers, across which the current must circulate before reaching the basolateral channels. This possibility was discounted by the measurements of the unidirectional fluxes. Only the ^{86}Rb fluxes were reduced by forskolin, not the Na or Cl fluxes. If the gap junctions were closed by forskolin (as well as the other Cl secretagogues), all fluxes should have been affected to a certain degree.

Although the net flux of ^{86}Rb and its reduction by forskolin did not quantitatively agree with the I_{sc} and its inhibition, this absence of agreement may be due to a lower permeability of the K channels to Rb compared to K. As previously indicated, Rb is not an identical replacement for K, particularly across K channels [11, 17, 32, 34]. This was evident across the RCE, when interchange between the cations resulted in a smaller I_{sc} across K channels when Rb replaced K.

Finally, our present results with microelectrodes (as well as previous ones) [8] indicate that a decrease in I_{sc} can only occur if the basolateral conductance, which mainly reflects the K permeability, decreases simultaneously with the increase in apical Cl permeability.

Why the RCE responds to Cl secretagogues in this peculiar way is not clear. Increasing apical Cl permeability will tend to thin the epithelium and move fluid in the stroma-to-tear direction. Closing of basolateral K channels will tend to accumulate KCl and swell the epithelium. Conditions that exacerbate tear evaporation with epithelial thinning could increase cellular cAMP, which would trigger K channel closure as a homeostatic mechanism. It is also possible that this mechanism may not play a role in normal conditions.

There are examples of K channels that are inhibited by cAMP [14, 15, 27, 29, 37], and evidence that channel closure is mediated by PKA phosphorylation has been obtained [26]. However, in most of these cases, this property serves a cell type-specific function. The exact role for such apparent cAMP-inhibited K conductance(s) in the rabbit cornea remains to be determined. As found before with other rabbit ocular epithelia, such as the ciliary body [35], lens [2] and conjunctiva [3], the physiology of the rabbit cornea exhibits particular characteristics, that differ from other species studied to date.

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