Biochimica et Biophysica Acta, 1026 (1990) 13–20
Elsevier

BBAMEM 74896

NPPB inhibits the basolateral membrane K^+ conductance in the isolated bullfrog cornea

Peter S. Reinach [1] and Howard F. Schoen [2]

[a] *Department of Ophthalmology, Washington University School of Medicine, St Louis, MO and* [2] *Department of Physiology, S U N Y -Health Science Center at Brooklyn, Brooklyn, NY (U S A)*

(Received 20 November 1989)
(Revised manuscript received 21 March 1990)

Key words Chloride channel inhibitor, Epithelium, Ouabain, Barium ion effect, Potassium ion conductance, (Cornea)

The effects of the Cl^- channel blocker, 4-nitro-2-(3-phenylpropylamino)benzoate (NPPB) on active transepithelial Cl^- transport were measured in the isolated bullfrog cornea. With a Cl^--free Ringers, stromal-side 10^{-5} M NPPB elicited a maximum depolarization of the membrane voltage from -72 ± 6 to -48 ± 9 mV ($n = 6$, $P < 0.05$) and reduced the magnitude of the depolarization induced by a 10-fold increase in K^+ concentration. Subsequent exposure to 10^{-4} M ouabain decreased the membrane voltage from -41 ± 6 mV to -25 ± 2 mV ($n = 6$, $P < 0.05$). After stimulation with 10^{-5} M amphotericin B of a short-circuit current, I_{sc}, largely accounted for by tear to stroma K^+ diffusion, this I_{sc} was effectively inhibited by 10^{-5} M NPPB on the stromal-side. This decrease reflected a fall in basolateral membrane K^+ conductance. In NaCl Ringers, inhibition of the essentially Cl^--originated I_{sc} either on the tear- or stromal-sides required instead 10^{-4} M NPPB. NPPB depolarized the membrane voltage from -55 ± 7 to -38 ± 6 mV ($n = 14$, $P < 0.05$). The direction of the change in the fractional apical membrane resistance (fR_o) depended upon its initial value; in those corneas with a lower value it increased whereas if they had a higher fR_o, 10^{-4} M NPPB consistently caused fR_o to fall. However, following exposure to $5 \cdot 10^{-3}$ M Ba^{2+} and a fall in fR_o, NPPB consistently caused fR_o to increase significantly from 30 ± 8 to $53 \pm 4\%$ ($n = 5$). Therefore, inhibition of active Cl^- transport by 10^{-4} M NPPB may be associated with declines in: (1) a basolateral membrane K^+ conductance that is distinct from a Ba^{2+}-sensitive pathway; (2) an apical membrane Cl^- conductance. Neither of these effects may be the result of a direct effect of NPPB on a conductance pathway because: (1) the drug was equipotent from either bathing solution; (2) following a one hour washout the I_{sc} had not fully recovered to its control value.

Introduction

The isolated bullfrog cornea transports Cl^- from the stromal to the tear-side bathing solution [1] The steps in this process include cellular Cl^- uptake across the basolateral membrane through a Na K $2Cl^-$ symport followed by exit across the apical membrane through conductive channels The driving force for Cl^- uptake is the chemical gradient for Na^+ between the bathing solution and the cell interior which is maintained by the activity of the Na^+/K^+ pump in the basolateral membrane The apical membrane is essentially Cl^- permselective but is also slightly conductive to K^+ [2] The basolateral membrane has an appreciable conductance to K^+ and is also somewhat conductive to Na^+ as well as other ions [3] K^+ is above electrochemical equilibrium because of Na^+/K^+ pump activity and therefore it recycles between the cell interior and the stromal-side This recycling in part establishes the driving force for Cl^- electrodiffusion across the apical membrane into the tears [4]

Diphenylamine-2-carboxylate (DPC) has been described as a Cl^- channel blocker because it decreased the apical membrane Cl^- conductance in the isolated bullfrog cornea [5] However, DPC was neither very effective nor potent and its effects were poorly reversible Furthermore, there exists some doubt about its selectivity because in the *Necturus* gallbladder (10^{-4} M

Abbreviations Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid, NPPB, 5-nitro-2-(3-phenylpropylamino)benzoate
Symbols I_{sc}, transcellular current under short-circuit conditions, g_t, transepithelial conductance, V_{sc}, intracellular voltage under short-circuit conditions, fR_o, fractional apical membrane resistance $= R_a/(R_a + R_b)$

Correspondence (current address) P S Reinach, Medical College of Georgia, Physiology and Endocrinology, Augusta, GA 30912, U S A

DPC inhibited Cl^-/HCO_3^- exchange [6] In other epithelia, a structural analogue of DPC, 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), is a more potent, effective and direct inhibitor of Cl^- conductance than DPC [7–14] For example, in the thick ascending limb of the loop of Henle (CTAL), the EC_{50} for decreasing the basolateral membrane Cl^- conductance was 8 10^{-8} M [7] We thought it possible that such high potency would be accompanied by an increased selectivity for inhibiting the apical membrane Cl^- conductance in the isolated bullfrog cornea Such a finding would make it meaningful to perform DC circuit analysis based on the effects of NPPB on the electrical parameters

We report on the effects of NPPB on the transepithelial and intracellular electrical parameters of isolated bullfrog corneas NPPB inhibited the essentially Cl^--originated short-circuit current, however, this decline was not the result of a selective decrease in a single conductance Therefore, it is not meaningful to use the effects of NPPB to perform DC circuit analysis because, in order for this approach to be valid, it is necessary to assume that an agent has a selective effect on a single conductance in the equivalent [2]

Materials and Methods

Bullfrogs were double pithed with a needle and the dissected corneas were immediately placed in either NaCl, Na_2SO_4 or sodium gluconate Ringers equilibrated with air [3,4] The corneas were mounted horizontally in a modified Ussing chamber for simultaneous measurements of their transepithelial and intracellular electrical parameters [3,4] We used a pH of 7 1 in this study because in preliminary experiments NPPB had larger and more reproducible effects than at a pH of 8 1 In some experiments, we isoosmotically mixed two of the three following modified Ringers (1–3) to impose a tear-to-stroma directed $[K^+]$ gradient (1) Na_2SO_4, (2) K_2SO_4 and (3) sucrose The tear-side mixture contained Na_2SO_4 and K_2SO_4 Ringers in a 2 1 ratio The stromal-side mixture contained Na_2SO_4 and sucrose in the same proportion Their respective compositions were (mM) (1) $Na^+ = 55$, $K^+ = 1\ 25$, $Ca^{2+} = 2\ 5$, Hepes = 5, $SO_4^{2-} = 58\ 75$ and sufficient sucrose was added to adjust the osmolarity to 220 mosM, (2) $K^+ = 56\ 25$, $Ca^{2+} = 2\ 5$, Hepes = 5, $SO_4^{2-} = 58\ 75$ and sucrose; (3) $K^+ = 1\ 25$, $Ca^{2+} = 2\ 5$, Hepes = 5, $SO_4^{2-} = 3\ 75$ and sucrose The tear-side mixture contained Na_2SO_4 and K_2SO_4 Ringers in a 2 1 ratio The stromal-side mixture contained Na_2SO_4 and sucrose also in a 2 1 ratio These mixtures established a $[K^+]$ diffusion gradient of 15 1 from tear to stroma Stock solutions containing 10 mM NPPB were prepared by dissolving the compound directly in the appropriate Ringers Precautions were taken to minimize drug exposure to light All the salts and sucrose were obtained from Fisher Scientific except Hepes (Sigma) and barium chloride (Mallinckrodt) Amphotericin B and ouabain were purchased from Sigma Chemical Co NPPB, 5-nitro-2-(3-phenylpropylamino)benzoic acid was generously provided as a gift by Dr Rainer Greger, Freiberg, F R G

All the data are expressed as means ± S E The level of significance was determined by paired Student's *t*-test

Results

The time dependent effects of 10^{-4} M NPPB in NaCl Ringers on the I_{sc} and V_{sc} are shown in the upper (A) and lower (B) panels of Fig 1, respectively In a typical experiment, tear-side exposure to NPPB resulted in a 81% decline of the I_{sc} after about 40 min During this time, the V_{sc} depolarized by 31 mV from −73 to −42 mV A transient tear-side substitution with Na_2SO_4 Ringers containing 10^{-4} M NPPB validated the impalement there was an additional depolarization of the V_{sc} which was reversible The inhibitory effects of 10^{-4} M NPPB were essentially the same regardless of its side of addition Washout of NPPB after 1 h resulted in a return to the control V_{sc} value, however, the I_{sc} only reached a value that was 45% of its control value Reexposure to tear-side 10^{-4} M NPPB caused after 20

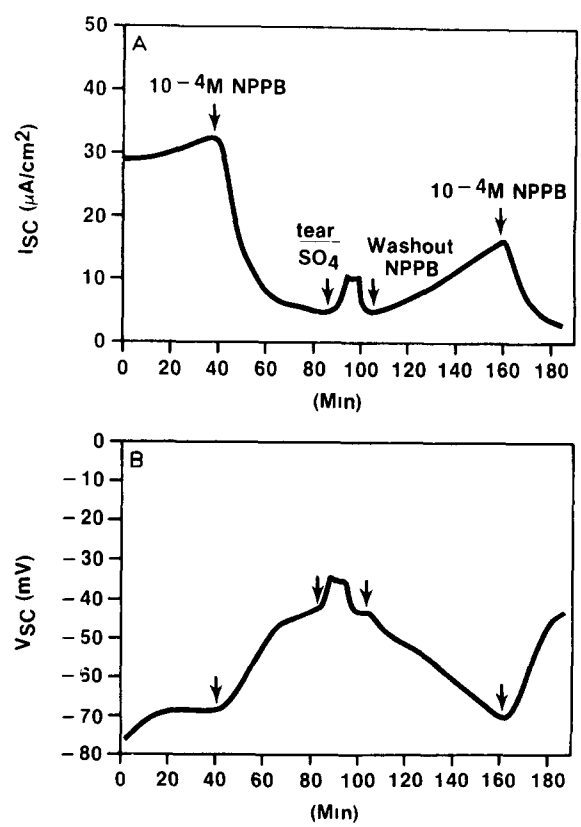

Fig 1 Time-dependent effects of tear-side 10^{-4} M NPPB in NaCl Ringers on the electrical parameters Top (A) and bottom (B) panels show the effects on the I_{sc} and V_{sc}, respectively

min the I_{sc} and V_{sc} to decline again to their previous inhibited values On the average in six experiments, 10^{-4} M NPPB (tear-side) decreased the I_{sc} by 75% (from 15 1 ± 2 1 to 3 8 ± 0 8 $\mu A/cm^2$) and decreased g_t from 0 50 ± 0.05 to 0 37 ± 0 05 mS/cm^2

Two different types of response of the fR_0 were seen to tear-side 10^{-4} M NPPB (cf Figs 2A and B) Note that in both A and B 10^{-5} M NPPB was without effect on either the tear or stromal-sides The impalement appeared adequate because tear-side substitution with Na_2SO_4 Ringers resulted in reversible depolarization of the V_{sc} and an increase in fR_0 In both groups, tear-side 10^{-4} M NPPB depolarized the V_{sc} but only in group A did it increase the fR_0 In contrast, with group B the typical response was a decrease in fR_0 The effect of tear-side 10^{-4} M NPPB on the V_{sc} for the two groups was a depolarization of the V_{sc} from -55 ± 7 to -38 ± 5 mV In group A, the fR_0 increased from 52 ± 6% to 75 ± 7% ($n = 8$, $P < 0.05$) whereas in group B the fR_0 decreased from 68 ± 9% to 41 ± 5% ($n = 6$, $P < 0.05$) These effects are not consistent with a selective decrease by NPPB of the apical membrane Cl^- conductance Such a decrease can only be associated with hyperpolarization of the V_{sc} and an increase in fR_0

The effects of stromal-side NPPB on the V_{sc} and fR_0 were measured in Na_2SO_4 Ringers to determine if NPPB affects other conductances besides Cl^- Under this condition, the I_{sc} is nearly zero because the apical membrane Na^+ conductance is insignificant [2] A typical recording showing the effect of 10^{-5} M NPPB on the I_{sc} is shown in the inset to Fig 3 The I_{sc} was 0 4 $\mu A/cm^2$ and was unaffected by NPPB Nevertheless, the Na^+/K^+ pump is functional because there is a significant net Na^+ tear to stroma flux [15] A typical record shown in Fig 3 indicates that 10^{-6} M NPPB was ineffective whereas 10^{-5} M NPPB elicited a maximal

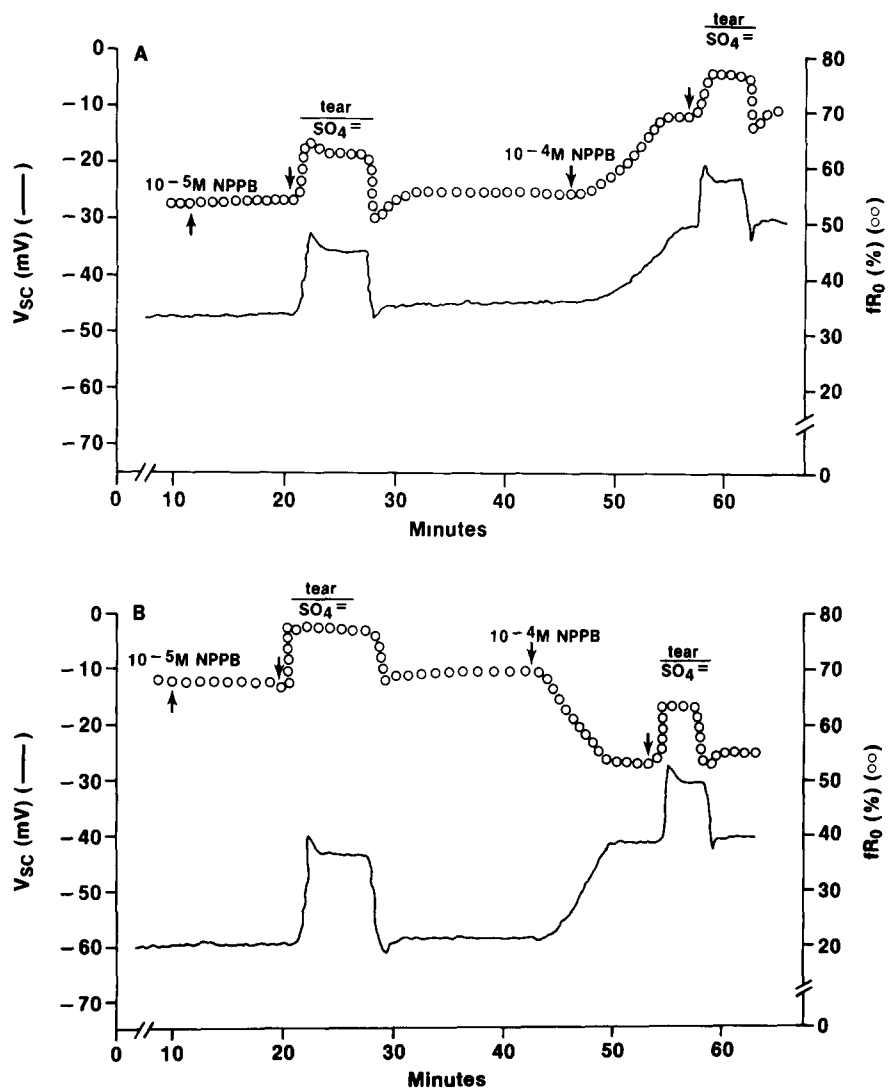

Fig 2 (A and B) Typical time course of effects of tear-side 10^{-4} M NPPB on intracellular electrical parameters Panel A shows the typical effects in a group of corneas ($n = 8$) in which NPPB depolarized the V_{sc} and increased fR_0 Panel B shows the typical effects in a group of corneas ($n = 6$) in which NPPB depolarized the V_{sc} but decreased fR_0

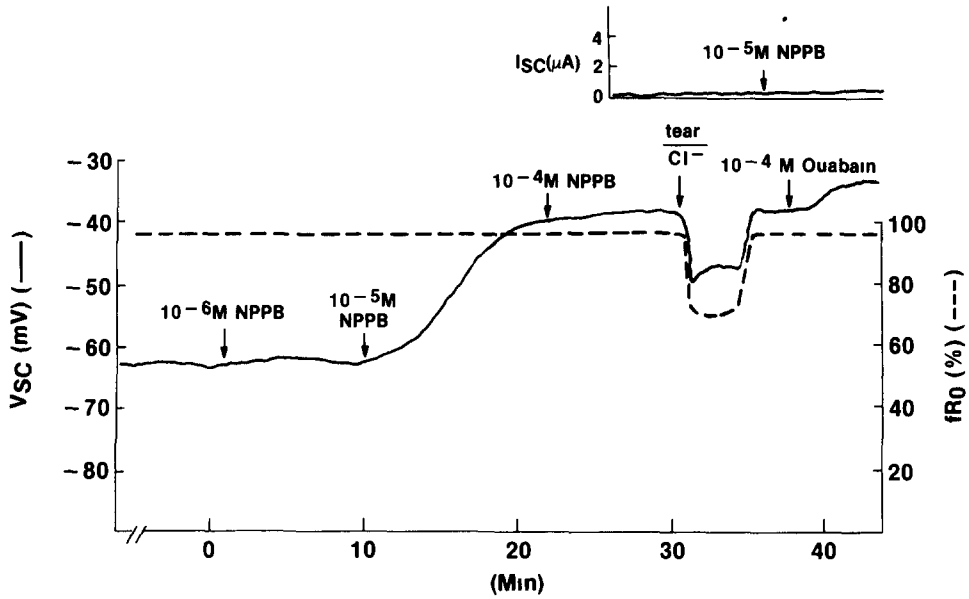

Fig 3 Typical time course of concentration-dependent effects of stromal-side NPPB on intracellular electrical parameters in Na_2SO_4 Ringers Following successive exposures to NPPB at concentrations from 10^{-6} to 10^{-4} M, a transient substitution of Na_2SO_4 with NaCl Ringers was performed These responses reflect a valid impalement The presence of Na^+/K^+ pump activity was assessed by measuring the effect of 10^{-4} M ouabain on the V_{sc}

change in the V_{sc} and no change in fR_o This discrepancy between the maximal effective concentration for depolarizing the V_{sc} in NaCl and Na_2SO_4 Ringers indicates that NPPB affects the conductance of a non-Cl^- pathway in the basolateral membrane whose sensitivity to NPPB is increased by the removal of Cl^- Also shown are the results of a validation procedure, namely, a transient substitution with NaCl Ringers which hyperpolarized the V_{sc} and decreased the fR_o Finally 10^{-4} M ouabain depolarized the V_{sc} by about 5 mV showing that NPPB is not an inhibitor of the Na^+/K^+ pump

In a Cl^--free (e g SO_4^{2-}) Ringers, fR_o can be greater than 90% because the apical membrane resistance exceeds that of the basolateral membrane by more than 10-fold Even relatively large changes in the basolateral membrane conductance have only a small effect on the fR_o To better assess the effect of NPPB on the basolateral membrane, the sensitivity of fR_o to detect an increase in basolateral membrane resistance was increased by incubating the tear-side of the cornea ($n = 5$) with 10^{-6} M amphotericin B This is a submaximal concentration which depolarized the V_{sc} from -92 ± 4 to -37 ± 3 mV, decreased the fR_o to $52 \pm 4\%$, increased the I_{sc} and g_t to 21 ± 2 $\mu A/cm^2$ and $0\,25 \pm 0\,01$ mS/cm^2, respectively, through an increase in the cation permselectivity of the apical membrane 10 μM NPPB (stromal-side) depolarized the V_{sc} to -22 ± 1 mV, significantly decreased the I_{sc} to 15 ± 1 $\mu A/cm^2$, the g_t to $0\,22 \pm 0\,01$ mS/cm^2 and the fR_o to $35 \pm 3\%$ Subsequent exposure to 10^{-4} M ouabain depolarized the V_{sc} by 7 mV to -15 ± 3 mV but it had no effect on the fR_o

In Fig 4 is an example of one of six determinations of the effects of a 10-fold increase in stromal-side $[K^+]$ to 25 mM in SO_4^{2-} Ringers on the V_{sc} and fR_o This change elicited a 36 mV depolarization of the V_{sc} and a small increase in fR_o Both of these changes were reversible following washout with 2 5 mM K^+ These effects are consistent with the previously reported appreciable basolateral membrane K^+ conductance [3,4] 10 μM NPPB (stromal-side) subsequently depolarized the V_{sc} to the same level as 25 mM K^+ With 10^{-5} M NPPB, 25 mM K^+ only depolarized the V_{sc} by about 10 mV suggesting that NPPB had partially blocked the basolateral membrane K^+ conductance but not the

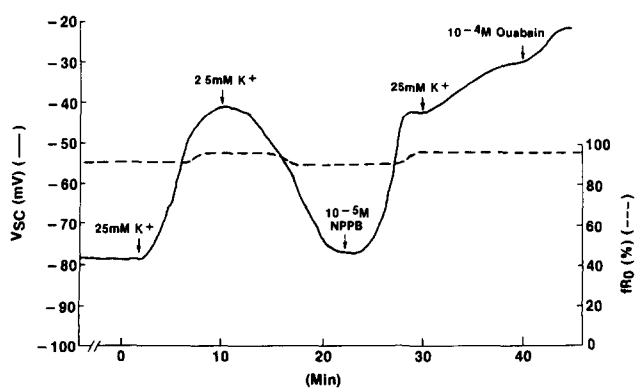

Fig 4 Typical time course of effects of stromal-side NPPB on intracellular electrical parameters in Na_2SO_4 Ringers Prior to NPPB exposure, the significant basolateral membrane K^+ conductance was validated based on the reversible change in the V_{sc} following a 10-fold increase in $[K^+]$ from 2 5 to 25 mM This substitution was subsequently performed with 10^{-5} M NPPB followed by exposure to 10^{-4} M ouabain

TABLE I

Consecutive effects of 25 mM K^+, 10^{-5} M NPPB and 10^{-4} M ouabain on V_{sc} in Na_2SO_4 Ringers (n = 6)

Values are given as means ± S E

Condition	V_{sc} (mV)
(a) Control (2 5 mM K^+)	−80±6
(b) 25 mM K^+	−42±6 *
(c) Control	−72±6
(d) NPPB	−48±9 *
(e) NPPB, 25 mM K^+	−41±6
(f) Ouabain	−25±2 *

* $P < 0\,05$ (with respect to previous period)

Na^+/K^+ pump (i e, ouabain depolarized the V_{sc}) A summary of these results for six corneas is provided in Table I A comparison of the effects of substitution with 25 mM K^+ on the average changes in the V_{sc} before and after exposure to 10^{-5} M NPPB show that the transference number for K^+ decreased from 0 66 to 0 12

To further document that NPPB is an inhibitor of the basolateral membrane K^+ conductance, corneas were exposed to a tear to stroma directed K^+ gradient (see Methods) and the cation permselectivity of the apical membrane was maximally increased with 10^{-5} M amphotericin B on the tear-side The resultant I_{sc} reflected Na^+/K^+ pump activity and K^+ diffusion The average for eight experiments of the concentration-dependent effects of NPPB on this I_{sc} are shown in Fig 5 At concentrations between 10^{-6} and 10^{-5} M, stromal-side NPPB had dose-dependent inhibitory effects on the I_{sc} With 10^{-5} M NPPB, the decline in the I_{sc} was about 80% of the increase elicited by amphotericin B

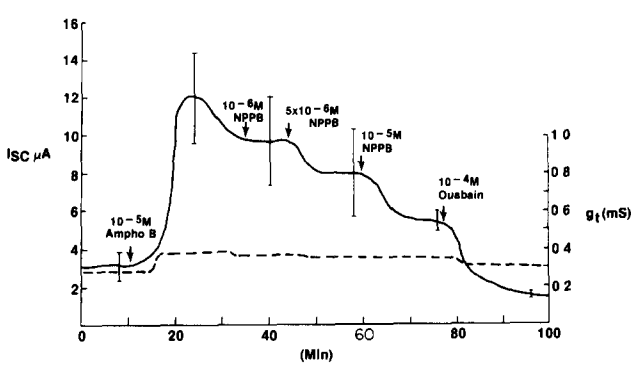

Fig 5 Time course of average concentration dependent inhibition by stromal-side NPPB of amphotericin B stimulated current elicited by tear to stroma K^+ diffusion Tear-side was incubated with a mixture in a 2 1 ratio of Na_2SO_4 and K_2SO_4 Ringers, respectively Stromal-side contained a mixture in a 2 1 ratio of Na_2SO_4 and sucrose Ringers The $[K^+]$ gradient directed from tear to stroma was 15 1 (i e, tear K^+ 37 5 and stroma K^+ 2 5 mM) In eight other corneas preincubated with 10^{-4} M ouabain, the amphotericin B stimulated current required to voltage clamp the transepithelial voltage to zero was 100% inhibited by 10^{-5} M NPPB on the stromal-side

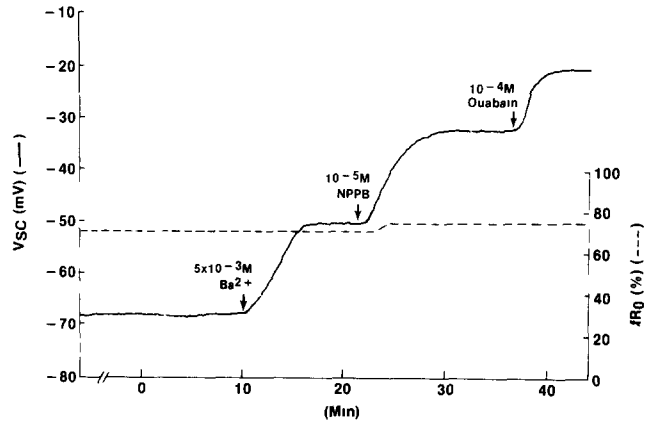

Fig 6 Time course of typical consecutive effects of stromal-side Ba^{2+}, NPPB and ouabain on intracellular electrical parameters in sodium gluconate Ringers Each succeeding compound was added together with the previous compound

The remaining I_{sc} was effectively inhibited by 10^{-4} M ouabain showing again that NPPB does not directly decrease Na^+/K^+ pump activity In six other experiments, the same protocol was followed except the corneas were preincubated with 10^{-4} M ouabain The amphotericin B stimulated I_{sc} was 100% inhibited by 10^{-5} M NPPB All of these results indicate that 10^{-5} M NPPB effectively decreases a basolateral membrane K^+ conductance

A barium-sensitive K^+ conductance was identified in the basolateral membrane of the corneal epithelium [4] To assess if this conductance is also NPPB sensitive, another series of experiments, an example of which is shown in Fig 6, was performed These experiments were performed in sodium gluconate Ringers to assure that any change in V_{sc} and fR_o essentially reflected an effect on a basolateral rather than an apical membrane conductance 5 mM Ba^{2+} (stromal-side) depolarized the V_{sc} by 18 mV without any significant change in fR_o 10 μM NPPB, in the presence of Ba^{2+}, further depolarized the V_{sc} by another 18 mV and slightly increased the fR_o These changes may in part reflect inhibition of a previously identified small apical membrane K^+ conductance [2] However, this change was not significant in five experiments (cf Table II) The V_{sc} depolarized by an additional 12 mV following exposure to 10^{-4} M

TABLE II

Effects of 5 10^{-3} M Ba^{2+}, 10^{-5} M NPPB and 10^{-4} M ouabain on intracellular electrical parameters in sodium gluconate Ringers (n = 5)

Values are given as means ± S E

	V_{sc} (mV)	fR_o (%)
Control	−76±2	87±3
Ba^{2+}	−53±3 *	81±3
NPPB	−33±4 *	79±3
Ouabain	−24±4 *	79±2

* $P < 0\,05$ with respect to previous period

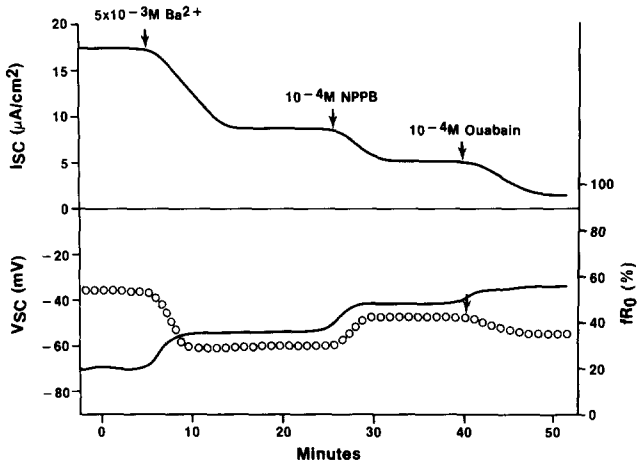

Fig 7 Time course of typical consecutive effects of stromal-side Ba^{2+}, NPPB and ouabain on transepithelial intracellular electrical parameters in NaCl Ringers In the top panel, is shown the I_{sc} The V_{sc} (———) and fR_o (○ ○ ○) are shown in the bottom panel Note that stromal-side 10^{-4} M NPPB was required to depolarize the V_{sc}, and it nearly reversed in all cases the decline in fR_o following exposure to Ba^{2+} alone

ouabain The additivity of their effects on the V_{sc} indicated in Table II shows that the basolateral membrane K^+ conductance is separable into Ba^{2+}- and NPPB-sensitive pathways

The experiment illustrated in Fig 6 was repeated but instead in NaCl Ringers to determine if preinhibition of a Ba^{2+}-sensitive K^+ conductance in the basolateral membrane would resolve any effect of NPPB on an apical membrane conductance A possible indication of an apical membrane interaction with NPPB is that under this condition NPPB would consistently increase fR_o A typical record of their individual effects on the I_{sc} and the intracellular electrical parameters are shown in the top and bottom panels of Fig 7, respectively The inhibitory effects of Ba^{2+} and NPPB on the I_{sc} and the V_{sc} were additive and Ba^{2+} significantly decreased the fR_o below its control value However, in contradistinction to Ba^{2+}, 10^{-4} M NPPB on the stromal-side consistently increased the fR_o and nearly restored it to its control level This increase could mean that NPPB has

TABLE III

Consecutive effects of 5 10^{-3} M Ba^{2+}, 10^{-4} M NPPB and 10^{-4} M ouabain electrical parameters in NaCl Ringers ($n = 5$)

Values are given as means ± S E

Condition	I_{sc} ($\mu A/cm^2$)	g_t (mS/cm^2)	fR_o (%)	V_{sc} (mV)
Control	17 3±2 2	0 26±0 03	56±2	−73±2
Ba^{2+}	8 8±1 3 *	0 24±0 03	30±8 *	−50±2 *
NPPB	5 6±2 0 *	0 22±0 01 *	53±4 *	−35±3 *
Ouabain	1 0±0 3 *	0 22±0 01	45±3	−29±3 *

* $P < 0\,05$ with respect to previous period

inhibitory effects on not only a basolateral but also an apical membrane conductance However, the interpretation of this increase in fR_o may be more complicated because the effects of tear-side substitution of Na_2SO_4 Ringers on the V_{sc} and fR_o were essentially unaffected by NPPB NPPB was not completely effective at either of these membranes because the overall decrease of the I_{sc} was 68% Ouabain (10^{-4} M) further inhibited the I_{sc} and depolarized the V_{sc} showing that neither Ba^{2+} or NPPB inhibit the Na^+/K^+ pump A summary of the results of five similar experiments is provided in Table III

Discussion

NPPB is a selective and direct inhibitor of Cl^- conductance in some tissues based on measurements of the effects of NPPB on either single channel activity or the intracellular electrical parameters [7–14] With bath-side application of NPPB to the thick ascending limb of the loop of Henle (CTAL), the EC_{50} for a decrease in the basolateral membrane Cl^- conductance was $8 \cdot 10^{-8}$ M based on increases in the fractional basolateral membrane resistance and hyperpolarization of the membrane voltage On the luminal side, the EC_{50} increased to 3 10^{-5} M At concentrations above 10^{-5} M, some of the less potent analogues of NPPB depolarized rather than hyperpolarized the membrane voltage which was interpreted as a nonselective effect in the CTAL [7] A basolateral membrane Cl^- conductance in the macula densa was identified based on hyperpolarization of the membrane voltage by luminally applied 10^{-5} M NPPB [14] In the thin ascending limb of the loop of Henle, NPPB addition to either side of the tissue decreased the relative permeability of Cl^- to Na^+ but its potency was dependent on its side of addition NPPB was more effective from the bath side on a molar basis and its EC_{50} was 3 10^{-5} M based on the assumption that its maximum inhibitory dose was 10^{-3} M [16] However, such an assumption may not be warranted, because in excised cell membrane patches of the rat exocrine pancreas, 10^{-4} M NPPB directly inhibited Ca^{2+} sensitive nonselective cation channels in the basolateral membrane [17] In cultured rat mesangial cells, another effect of NPPB has been identified in the same concentration range at which it is a selective inhibitor of Cl^- conductance in other tissues [18] Between 1 and 10 micromolar it was a potent inhibitor of prostonoid biosynthesis 8 μM NPPB inhibited prostaglandin E_2 release by 50% Therefore, with any use of NPPB and its related compounds it is first necessary to ascertain their effectiveness and selectivity before using them to derive conclusions about mechanisms of ion transport

In the cornea, NPPB had effects on the intracellular electrical parameters over the same concentration range

at which it selectively decreased a Cl^- conductance in some other tissues However, its effects in the cornea did not reflect a direct decrease in apical membrane Cl^- conductance NPPB consistently depolarized the V_{sc} in both Cl^--free and NaCl Ringers and had variable effects on the fR_o All of these effects are not consistent with a direct and selective inhibition of a Cl^- conductance because such an effect requires a hyperpolarization of the V_{sc} and an increase in fR_o

In those tissues in which NPPB selectively decreased Cl^- conductance, the potency of NPPB was affected by its side of addition whereas in the cornea it was equipotent This side dependence is a reflection of NPPB's selectivity because of its accessibility to the membrane with which it interacts Presumably this accessibility is greater from the bath facing the membrane containing the conductance with which it interacts In the cornea, NPPB may be equipotent from either bath because NPPB is directly modifying an intracellular regulator of these as well as other conductances For example, if NPPB inhibited prostanoid biosynthesis in the cornea, as described in cultured rat mesangial cells, this change may have effects on a number of parameters

The effects of NPPB were measured on the electrical parameters in Cl^--free Ringer's solutions to determine if any change in the basolateral membrane conductance could account for inhibition of the essentially Cl^--originated I_{sc} Such a change could explain the inhibition because the electrical driving force for Cl^- efflux across the apical membrane into the tears stems in large part from K^+ recycling between the stromal-side bath and the cell interior across the basolateral membrane [4] One of these pathways is Ba^{2+} sensitive because in NaCl Ringers Ba^{2+} depolarized the V_{sc}, inhibited the I_{sc} and decreased fR_o In Cl^--free (gluconate) Ringers, 10^{-5} M NPPB was maximally effective in depolarizing the V_{sc} in Na_2SO_4 and sodium gluconate Ringer's solutions Inhibition by NPPB occurred across a pathway in parallel with the Ba^{2+}-sensitive pathway because their depolarizing effects were additive in NaCl and sodium gluconate Ringers Further indications of an effect by 10^{-5} M NPPB on a basolateral membrane K^+ conductance in SO_4^{2-} Ringers include (1) a significant decrease in g_t of 12% following exposure to 10^{-6} M amphotericin B and (2) a 82% decrease in the transference number for K^+ (cf Table I) Therefore inhibition of active Cl^- transport is in part explained by a decrease in a basolateral membrane K^+ conductance that is distinct from the Na^+/K^+ pump and the Ba^{2+}-sensitive pathway

Another approach to determine a basolateral membrane interaction with NPPB was to measure its effects on the electrical parameters in Cl^--free Ringers following incubation with amphotericin B This procedure permits easier resolution of any NPPB interaction with the basolateral membrane because amphotericin B increased the cation permselectivity of the apical membrane Even though amphotericin B alters intracellular ion composition and depolarizes the V_{sc}, which could result in changes in cell pH and volume and basolateral membrane conductance, none of these effects compromise Na^+/K^+ pump function because the I_{sc} stimulation is sustained and stable Despite any changes in the baseline membrane conductance, this technique still permitted identification of any qualitative changes in the basolateral membrane resistance based on the direction of the change in fR_o following exposure to NPPB Following exposure to a submaximal concentration of amphotericin B (i e, 10^{-6} M), fR_o decreased 10 μM NPPB was maximally effective in further decreasing fR_o and depolarizing the V_{sc} This effect of NPPB coupled with its dose-dependent decrease of the amphotericin B stimulated I_{sc}, accounted for in large part by tear to stroma K^+ diffusion, further suggest that NPPB increases the resistance of a K^+ pathway in the basolateral membrane

The dose dependent relationship for inhibition of active Cl^- transport by NPPB is remarkably steep because 10^{-5} M NPPB was without effect whereas 10^{-4} M NPPB was maximally effective This finding suggests that the pathways in the basolateral membrane affected by NPPB are also remarkably sensitive to chloride It is not possible to identify how the presence of Cl^- decreases the effect of NPPB on the basolateral membrane conductance An even more profound anion-dependent effect on the sensitivity of a basolateral membrane K^+ conductance to Ba^{2+} has been described in the isolated toad and rabbit urinary bladders [19,20] In these tissues, this conductance's sensitivity to Ba^{2+} disappears following the substitution of NaCl with sodium gluconate Ringers

Even though NPPB consistently depolarized the V_{sc}, it had variable effects on the fR_o as shown in Fig 2 It is possible that NPPB elicited in corneas with a higher fR_o (i e, $R_a > R_b$) a larger increase in the basolateral membrane resistance which caused fR_o to fall Conversely, if the fR_o was lower (i e, $R_b > R_a$), then NPPB elicited a relatively larger increase in the apical membrane resistance which instead caused fR_o to increase In contradistinction, after exposure to Ba^{2+}, NPPB consistently increased the fR_o perhaps because the increase in the apical membrane resistance was larger than that of the Ba^{2+}-blocked basolateral membrane However, there exists some doubt as to whether or not NPPB increased the apical membrane resistance because the presence of NPPB did not appear to affect any of the changes in the V_{sc} and fR_o following a transient tear side substitution of NaCl with Na_2SO_4 Ringers This uncertainty may stem from the possibility that NPPB's effects on conductances and net Cl^- transport are an indirect effect If this is the case, an effect

by NPPB on an intracellular regulator may cause more changes than those accounted for by this study

Acknowledgements

We thank Dr Rainer Greger and Hoechst Pharmaceutical for their gift of NPPB This work was supported by National Institutes of Health Grants EY-04795 (to P Reinach) and RO1-DK33612 The technical assistance of F Akata is deeply appreciated

References

1 Zadunaisky, J A (1966) Am J Physiol 211, 506–512
2 Reuss, L, Reinach, P, Weinman, S and O'Grady, T P (1983) Am J Physiol 244, C336–C347
3 Reinach, P, Thurman, C and Klemperer, G (1987) J Membr Biol 99, 205–213
4 Reinach, P and Nagel, W (1985) J Membr Biol 87, 201–209
5 Scheide, J I and Zadunaisky, J A (1988) Am J Physiol 254, C519–C525
6 Reuss, L, Constantin, J L and Bazile, J E (1987) Am J Physiol 253, C79–C89
7 Wangermann, P, Witter, M, Di Stefano, A, Englert, H C, Lang, J, Schaltter, E and Greger, R (1986) Plfuegers Arch 407, S128–S141
8 Hayslett, J P, Gogelein, H, Kunzelmann, K and Greger, R (1987) Pfluegers Arch 410, 487–494
9 Greger, R, Schlatter, E and Gogelein, H (1987) Pfluegers Arch 409, 114–121
10 Stutts, M J, Henke, D and Boucher, R C (1987) Fed Proc 46, 1236
11 Landry, D W, Reitman, M, Cragoe, E J, Jr and Al-Awqati, Q (1987) J Gen Physiol 90, 779–798
12 Breuer, W (1989) J Membr Biol 107, 36–42
13 Novak, I and Greger, R (1988) Pfluegers Arch 411, 546–553
14 Schlatter, E, Salomonsson, M, Persson, A E G and Greger, R (1989) Pfluegers Arch 414, 286–290
15 Candia, O A and Askew, W A (1968) Biochim Biophys Acta 163, 262–265
16 Isozaki, T, Yoshitomi, K and Imai, M (1989) Am J Physiol 257, F92–F98
17 Gogelein, H and Pfanmuller, B (1988) Pfluegers Arch 413, 287–298
18 Breuer, W and Skorecki, K (1989) Biochem Biophys Res Comm 163, 398–405
19 Lewis, S A, Butt, A G, Bowler, J M, Leader, J P and McKnight, A D C (1985) J Membr Biol 83, 119–138
20 Donaldson, P J, Chen, L K and Lewis, S A (1989) Am J Physiol 256, F1125–F1134