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Rabbit Corneal Hydration and the Bicarbonate Pump

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Abstract. Experiments were conducted on the transport properties of the rabbit corneal endothelium at 22 °C, at which temperature the endothelium was able to stabilize the hydration of corneal stroma at physiological values. When bicarbonate was omitted from the bathing solution, the cornea swelled at 11 ± 1 μm.h^{-1}. The swelling was completely reversible upon the subsequent re-introduction of bicarbonate. Similar swelling rates were observed when the endothelial pump was irreversibly inhibited with ouabain. In an Ussing-type chamber, the endothelium developed an electrical resistance of 25.0 ± 1.0 Ω.cm^2 and a short circuit current (s.c.c.) of 6.0 ± 1.1 μA.cm^{-2}. Neither electrical resistance of the corneal endothelium nor its s.c.c. were changed significantly after exposure to 0.5 mM amiloride. Ouabain abolished the s.c.c. but had no significant effect on resistance. When paired preparations were short-circuited, the endothelium developed a net $H[^{14}C]O_3^-$ flux of 0.24 ± 0.03 μmoles.cm^{-2}.h^{-1} into the aqueous humour, which was close in magnitude and direction to the s.c.c. of 0.22 ± 0.01 μEq.cm^{-2}.h^{-1}. There was no significant net flux of ^{86}Rb (0.04 ± 0.03 μmoles.cm^{-2}.h^{-1}). Similar magnitude fluxes for both bicarbonate and rubidium were found with open-circuit preparations. It is suggested that a metabolically driven electrogenic bicarbonate current passing across the corneal endothelium is solely responsible for maintaining corneal hydration at 22 °C. Based on these and other studies, a model is proposed for active bicarbonate transport across corneal endothelium consisting of uphill entry into the cell through a baso-lateral membrane sodium/bicarbonate cotransporter (NBC) and downhill exit through an apical membrane anion channel. Studies on the transport properties of the endothelium at 35 °C are discussed and reasons suggested for the discrepancy between short circuit current and net bicarbonate flux at this closed eye temperature.

Key words: Corneal endothelium — Bicarbonate pump — Sodium/bicarbonate cotransporter — Ussing chamber

Introduction

The corneal stroma, a connective tissue that comprises about 90% of the mammalian cornea, continually strives to swell. If it does swell, it loses its optical transparency. The stromal swelling tendency is neutralized by the presence of a 'pump' in the endothelial monolayer lining the posterior surface of the stroma, which continuously drives osmotica from the stroma into the bathing solution. This 'pump' is dependent on the presence of Cl^- and HCO_3^-, and requires a number of transport proteins including basolateral $Na^+/2HCO_3^-$ cotransport, $Na^+/K^+/2Cl^-$ cotransport, $C1^-/HCO_3^-$ exchange and apical anion channels permeable to both Cl^- and HCO_3^- (for a recent, comprehensive review *see* Bonanno, 2003). The relationship for a dynamically stable cornea is given by;

$$J_p = \frac{\omega.\Delta\gamma}{\sigma} \quad (1)$$

The left-hand side of the equation represents the 'pump' and the right-hand side represents the equal magnitude 'leak' of those osmotica back across the monolayer. J_p is the rate at which osmotica are pumped out of the stroma, and ω and σ are, respectively, the permeability and reflection coefficient of the monolayer to the 'pumped' solute and $\Delta\gamma$ is the gel pressure of the corneal stroma, which continuously drives material inwards. The continuous cycling of the osmotica across the endothelium is essential for the maintenance of a transparent cornea. The identity

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of the cycling osmotica is of interest but not yet fully understood.

It is generally agreed that at 35 °C a major component of the pump is the bicarbonate ion (Hodson & Miller, 1976; Jentsch et al., 1984; Bonanno & Gissan, 1992a, b; Riley et al., 1995, Bonanno, 2003). However, at 35 °C there are clearly other ionic currents flowing across the corneal endothelium because the net bicarbonate flux is always significantly smaller than the short-circuit current (s.c.c.) by 30–50% (Wigham & Hodson, 1985). The 'missing' net flux (or fluxes) across short-circuited rabbit corneal endothelium at 35 °C is reported to be neither lactate nor any other metabolite derived from glucose (Riley et al., 1977) nor Na^+ (Wigham & Hodson, 1985) and has not, to the present, been identified. The 'missing' current may indicate the presence of an osmotic flux, which could contribute towards the regulation of corneal hydration. With the current capability of genetic modification of the proteins of the cornea for potential clinical treatment (Wang et al., 2000) it has become more important to define unambiguously the transport system(s) in the corneal endothelium, which regulate(s) corneal hydration, and to check whether ion fluxes other than bicarbonate contribute to the maintenance of corneal transparency. We therefore re-visited the problem (Fischbarg & Lim, 1974; Lieboritch & Fischbarg, 1982–83; Jentsch et al., 1984; Bonanno & Giasson, 1992 a, b; Wigham et al., 1996). Candidates for the 'missing' flux are hydrogen ions, potassium ions (Kuang et al., 2001) and, possibly, chloride ions. The presence of chloride ions is certainly necessary for the operation of the endothelial 'pump' (Winkler et al., 1992), but the recent characterization of a chloride-dependent sodium/bicarbonate co-transporter in the plasma membrane of corneal endothelial cells (Lane, Wigham & Hodson, 2000) might explain the chloride dependence of the endothelial transporters. Net chloride fluxes have not been found across short-circuited rabbit corneal endothelium (Hodson & Miller, 1976).

We wished to test this suggestion that the 'missing' flux might be generated by potassium or hydrogen ions. Direct measurement of uni-directional hydrogen fluxes was impractical because of the very high exchange of water through this tissue (Hodson & Wigham, 1987) facilitated by water channels (Li et al., 1999). It has been proposed that the Na^+/H^+ exchanger (NHE) acts in tandem with carbonic anhydrase through a pathway that could have a regulatory role on endothelial transport via its effect on Na^+ re-entry (Lieboritch & Fischbarg, 1982–83). If this suggestion were true, then it would be expected that reduction of NHE activity might correspondingly reduce the difference between the net bicarbonate flux and s.c.c.. Unfortunately, direct NHE inhibition by any of the family of inhibitors (amiloride, or its dimethyl, ethyl, isopropyl, hexamethylene, methyl, or isobutyl derivatives) not only reduces short-circuit current but also produces a progressive increase in transendothelial permeability at 35 °C sufficient to make stable flux studies impractical. We could think of no further experimental procedures that might investigate this suggestion at 35 °C.

We pursued our aim by exploiting the high tolerance to lowered temperature variation shown by the cornea (Hodson, 1975). The endothelium is able to maintain the cornea at its physiological hydration at temperatures down to 20°C (Hodson, 1975). As the corneal temperature lowers, the ''pump'' is diminished sufficiently to compensate for the decrease in the endothelium's permeability. We monitored corneal hydration for its dependence on bicarbonate at this temperature and, separately, we monitored the transport characteristics of $H[^{14}C]O_3^-$ at the same time as ^{86}Rb across the open and short-circuited corneal endothelium in an Ussing-type chamber at 22 °C. We re-examine data of corneal endothelium transport properties at 35 °C and propose a model of the 'missing' net flux.

Materials and Methods

New Zealand white rabbits 3–4 months old were killed by intravenous injection of sodium pentabarbitone whilst they were calm and relaxed. Their eyes, complete with conjunctiva and lids, were dissected (Dikstein & Maurice, 1972) and the epithelium was removed from the apical surface with rotating bristle brushes.

Specular microscope studies at 35 and 22 °C

Rabbit corneas were pre-swollen in whole eyes by refrigeration at 4 °C overnight and the next day, the cornea, together with a scleral rim, were dissected and mounted under the specular microscope by the method of Dikstein and Maurice (1972) and perfused at 2 ml/h with normal rabbit ringer (NRR) solution (in mM: NaCl 106, $NaHCO_3$ 21, N-[2-hydroxyethyethyl]piperazine-N′-[2-ethanesulfonic acid] (HEPES) 20, KCl 6.7, Na_2HPO_4 5.55, glucose 4.45, $CaCl_2$ 1.4, reduced glutathione 1, $MgSO_4$ 0.6 adjusted to pH 7.4. Corneal thickness was monitored at 35 °C or at room temperature (22 ± 2 °C) with the specular microscope until they stabilized (n = 3 pairs).

In other experiments, fresh corneas were perfused with NRR at 22 °C for 3 h and after corneal thickness stabilization, they were perfused with bicarbonate-free ringer (BCFR) solution (in mM: NaCl 127, N-[2-hydroxyethyethyl]piperazine-N′-[2-ethanesulfonic acid] (HEPES) 20, KC1 6.7, Na_2HPO_4 5.55, glucose 4.45, $CaCl_2$ 1.4, reduced glutathione 1, $MgSO_4$ 0.6) adjusted to pH 7.4 at 2 $ml.h^{-1}$ for 2 h, after which they were re-substituted back to NRR for a further 3 h. To facilitate rapid solution changes, changes of solution were initiated by flushing 4 ml through the chamber (volume 1.2 ml) over a 5 minute period. Control experiments showed this rapid flushing alone had no detectable effects on corneal thickness. In other experiments, fresh preparations were mounted under the specular microscope at either 35 °C or at room temperature (22 ± 2 °C) until they stabilized and then had their endothelial transport activities inhibited by the addition of 5×10^{-4} M ouabain added to the perfusing NRR pairs of eyes after 2 h (n = 3 pairs) or 5 h (n = 3 pairs).

Transendothelial Electrical Measurements

Tissue preparation and mounting of de-epithelialized corneas between two halves of a modified Ussing chamber were performed as described previously (Hodson & Wigham, 1983; both half-chambers and their line supplies were sealed from the atmosphere. Chamber solution stirring was effected by the action of external rotating magnets on rotating stainless steel propellers mounted on jeweller's bearings in each half chamber. The effectiveness of the system in retaining bicarbonate/CO_2 was checked by perfusing each half-chamber with radioactive bicarbonate included in the Ringer when the half chambers were separated from each other by an impermeable neoprene membrane. In both half-chambers exudate activity of radioactive bicarbonate/CO_2 equalled infusate activity within the experimental error of 1%, indicating that each half-chamber was effectively sealed from CO_2 loss to atmosphere. Further checks were carried out to detect any innate directionality of the system by perfusing one half-chamber with radioactive-loaded Ringer and noting the exudates' activity from the opposite half-chamber as the isotope passed across a Visking dialysis membrane that separated the half-chambers. We observed no innate directionality in the system; trans-dialysis membrane exudate activities from either side were equal in magnitude. Chambers were perfused at 10 ml/h with NRR solution. Tissue temperatures of 22.0 ± 0.5 °C were achieved by immersion of a DC1 Dip Cooler (Nickel Electro Ltd, Somerset, UK) in the silicon oil bath surrounding the perfusion chamber. Higher temperatures were achieved by adjusting the oil bath. Each preparation was allowed to equilibrate both its spontaneous electrical potential and resistance. The series resistance of the de-endothelialized stroma and NRR changed very slightly with temperature and the appropriate values were subtracted in calculating the endothelial resistance from the total measured resistance.

Flux Measurements

Chambers were perfused with NRR solution containing 1.16 MBq of ^{86}Rb and 3.7 MBq of $H[^{14}C]O_3^-$ isotopes. In order to minimize the number of animals used, preliminary experiments characterizing the clearance properties of radioisotopes in the chamber were conducted with Visking dialysis tubing separating the two half-chambers and estimating the magnitudes of the systematic and random errors. From our expectations of the smaller magnitudes of the fluxes than those we had measured previously (Hodson & Wigham, 1987), it became clear that it would not be practical to reverse the direction of the fluxes, as before, because the small slowly emptying reservoirs in each half-chamber had a significant effect on these (lower) flux rates, and so we chose to study unidirectional fluxes simultaneously in paired eyes. From the estimated errors, it seemed that four pairs of eyes would be sufficient to measure the unidirectional bicarbonate and rubidium fluxes to an accuracy of around 8% and this was sufficient to distinguish this data from previous estimates at 35 °C where the net bicarbonate fluxes and short-circuit current differed by 20–40%. Ion fluxes were measured whilst the Ussing chambers were kept at 22.0 ± 0.5 °C, regulated by a separate dip cooler in each silicon oil bath. Paired corneas were mounted simultaneously and allowed to stabilize for 1 h. Three resistance measurements were then taken at 6 min intervals and the short-circuit current generated by the endothelium calculated, taking into account, for these low-resistance membranes, the series chamber resistance (for a more detailed description *see* (Wigham & Hodson, 1981). This current was then applied to the preparation by the current generator for a further 20 min. The outflow of NRR, containing the isotopes, was collected at 6 min intervals from both half-chamber outlets for 2 h. The shorting current was then removed and the PD_e and R_t re-measured to assess any changes in the short-circuit current. In this series they were always negligible. The endothelium was then removed from the corneas in situ (Wigham & Hodson, 1981) and the contribution of the remaining corneal stroma to the impedance of the isotopes was then measured. Samples were collected for a further 30 min after an equilibration period of 20 min. The blank resistance was then re-confirmed. Sample activity was measured using a scintillation counter (Wallac 1409). The two different energy emission spectra of ^{86}Rb and ^{14}C were ascertained in preliminary experiments (^{86}Rb maximum β-emission 1.77 MeV, ^{14}C maximum β-emission 0.158 MeV), enabling two windows to be set on the scintillation counter to quantify the two isotopes independently. The majority of the ^{14}C energy spectrum falls between 1–300 and the majority of the ^{86}Rb between 700–1024. These two ranges were therefore used to measure the CPM rate of the two isotopes. 1–300 was designated the lower, carbon window and 700–1024, the upper or rubidium window. Due to the skewed distribution of the energy curves for the two isotopes the upper, rubidium window was "pure" and did not contain any ^{14}C counts, but the carbon window contained a small percentage of ^{86}Rb. The number of ^{86}Rb counts in the lower carbon window is called the "crossover' (typically 2% of the total in that window) and is measured prior to the mixing of the two isotopes and subsequently subtracted from the carbon window counts to give a pure ^{14}C CPM rate. The background and crossover of the ^{86}Rb into the ^{14}C window were calculated and subtracted. The difference between the two directions of flux ($J_{(lens\text{-}tear)}$-$J_{(tear\text{-}lens)}$), corrected for preparation area, gave the net flux ($\mu mol.cm^{-2}.h^{-1}$). The flux measurements were repeated in open-circuit conditions (n = 4 pairs). In the case of bicarbonate/CO_2 determinations we were unable to distinguish the relative proportions of either component in the net fluxes but there is direct evidence for uphill transport of bicarbonate in these cells via the NBC activity in the basal membrane (Jentsch et al., 1984, Bonanno & Giasson 1992, Lane et al., 2000) and there is apparently no evidence for CO_2 transport across any tissue other than via passive processes. We assumed therefore that any net transport of bicarbonate/CO_2 across this tissue arose from the bicarbonate component.

Amiloride Inhibition Studies as a Function of Temperature

De-epithelialized corneal preparations were allowed to equilibrate to their spontaneous electrical potential and resistance at 22, 25, 30 or 35 °C (n = 3) when 0.5 mM amiloride was added and the changes to s.c.c. and resistance noted. The appropriate blank resistance values were subtracted in calculating the endothelial resistance as described above.

Results

Specular Microscope Studies at 35 and 22 °C

When paired corneas were refrigerated overnight, then mounted under a specular microscope, they exhibited the classical temperature reversal phenomenon (Davson, 1995) and over a period of time deturgesced to near their original physiological thickness. At 35 °C the stroma deturgesced from 560 to 418 μm (mean; n = 3) over a period of 5 h at a maximal rate of around 40 $\mu m.h^{-1}$ (Fig. 1). The other

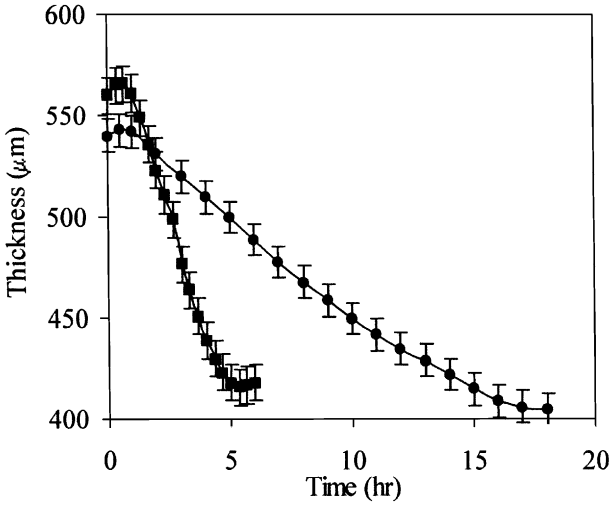

Fig. 1. The deturgescence of rabbit corneal endothelium at 22 (*filled circles*) and 35 (*filled squares*) ± 2 °C of pre-swelled corneas, measured by specular microscopy ($n = 3$).

cornea from each pair was perfused at 22 °C under the specular microscope. The initial mean stromal thickness of 540 μm slowly reduced to a stabilized mean value of 405 μm over 17 h. At 22 °C the cornea deturgesced at around 10 $\mu m.h^{-1}$ (Fig. 1).

At 22 °C, fresh de-epithelialized corneas stabilized, but upon removal of bicarbonate/CO_2 from the solution they swelled at 11 ± 1 μmh^{-1} ($n = 3$). On subsequent replacement of bicarbonate/CO_2 to the Ringer, the corneas initially deturgesced at 10.5 ± 1 μmh^{-1} (Fig. 1), eventually to return to their original hydration. When the endothelial transport system was inhibited with ouabain at 22 °C, the corneas swelled at 10 ± 1 $\mu m.h^{-1}$ ($n = 3$) and this swelling was essentially irreversible. The corneal swelling rates in bicarbonate/CO_2–free solutions (BCFR) and in ouabain were not significantly different.

Flux Measurements

The mean unidirectional flux of $H[^{14}C]O_3^-$ across the cornea from tear-side to lens-side at 22 °C was 1.23 ± 0.03 $\mu Eq.cm^{-2}.h^{-1}$ ($n = 4$). The mean unidirectional flux of $H[^{14}C]O_3^-$ from lens-side to tear-side at 22 °C was 0.99 ± 0.03 $\mu Eq.cm^{-2}.h^{-1}$ ($n = 4$). From these data, the net $H[^{14}C]O_3^-$ flux at 22 °C across the cornea was calculated as 0.24 ± 0.03 $\mu moles.cm^{-2}.h^{-1}$ from tear-side to lens-side (Table 1). The mean unidirectional flux of ^{86}Rb across the de-epithelialized corneal preparation from tear-side to lens-side at 22 °C was 0.29 ± 0.02 $\mu Eq.cm^{-2}.h^{-1}$ ($n = 4$). The mean unidirectional flux of ^{86}Rb from lens-side to tear-side at 22 °C was -0.33 ± 0.03 $nEq.cm^{-2}.h^{-1}$($n = 4$). There was no significant ($P > 0.05$) net ^{86}Rb flux across the cornea at 22 °C (0.04 ± 0.03 $\mu moles.cm^{-2}\ h^{-1}$ from lens-side to tear-side) (Table 1).

Whilst the isotopic flux data was collected from each preparation, the s.c.c. generated by the corneal endothelium was measured. The mean s.c.c. ($n = 8$) at 22 °C was 0.22 ± 0.01 $\mu Eq.cm^{-2}.h^{-1}$ (5.91 ± 0.29 μAcm^{-2}). The net $H[^{14}C]O_3^-$ flux across each corneal preparation was approximately equal to the s.c.c. (Fig. 2). There was negligible net ^{86}Rb flux when the endothelium was generating this same s.c.c. at 22 °C (Fig. 2).

Amiloride Inhibition Studies as a Function of Temperature

The temperature dependence of both total short-circuit current passing across the rabbit corneal endothelium and the residual current after inhibition by 0.5 mM amiloride is shown in Fig. 3*A*. At 22.0 ± 0.5 °C the s.c.c. generated by the endothelium was 6.0 ± 1.2 $\mu A.cm^2$. With the addition of 0.5 mM amiloride to the NRR the s.c.c. showed no significant change. The presence of 0.5 mM amiloride had no additional effect on the tissue resistance of 25.0 ± 1 $\Omega\ cm^2$ at 22.0 °C (Fig. 3*B*). Amiloride-inhibited tissue gave stable readings for at least 30 minutes.

Discussion

At 22 °C, we confirmed that the corneal endothelium is able to stabilize corneal hydration by an active transport process. The active transport is completely inhibited in the absence of bicarbonate ions (the swelling rate in bicarbonate free solutions is not significantly different from the swelling rate in ouabain Fig. 1); and in both cases the permeability of the endothelium is unchanged). The inhibition of the transport process by bicarbonate removal is completely reversible (Fig. 1). The same two properties (total transport inhibition in bicarbonate-free solutions and reversibility) are also exhibited by the endothelium at 35°C (Wigham & Hodson, 1981).

The uni-directional bicarbonate and rubidium fluxes are similar in magnitude in both open- and short-circuit condition (Fig. 2). The probable explanation for this resides in the non-permselective properties of the endothelial barrier (Hodson & Wigham, 1983). Ions permeate across the corneal endothelial monolayer at relative rates determined by the product of their concentrations and their free diffusion coefficients in accordance with Hodgkin's law. It would be expected that the neutralizing passive current in the open-circuit condition would primarily be carried by solution ions in the highest concentrations, i.e., Na^+ and Cl^-. From Hodgkin's law, it is estimated that the net flux for bicarbonate would be expected to decrease by about 6% in open

Table 1. Rabbit corneal endothelium flux data for ^{14}C and ^{86}Rb at 22°C

	$J_{(tear\text{-}lens)}$ ($\mu moles.cm^{-2}(h^{-1})$)		$J_{(lens\text{-}tear)}$ ($\mu moles.cm^{-2}(h^{-1})$)		Net Flux ($\mu moles.cm^{-2}.h^{-1}$)	
	^{14}C	^{86}Rb	^{14}C	^{86}Rb	^{14}C	^{86}Rb
Mean	1.23	0.29	0.99	0.33	0.24	−0.04
SEM	0.03	0.02	0.03	0.03	0.03	0.03

Shown are the mean flux data ($n = 4$) for $J_{(lens\text{-}tear)}$ and $J_{(tear\text{-}lens)}$ and the flux rate for ^{14}C and ^{86}Rb at 22 ± 0.5 °C for rabbit corneal endothelium.

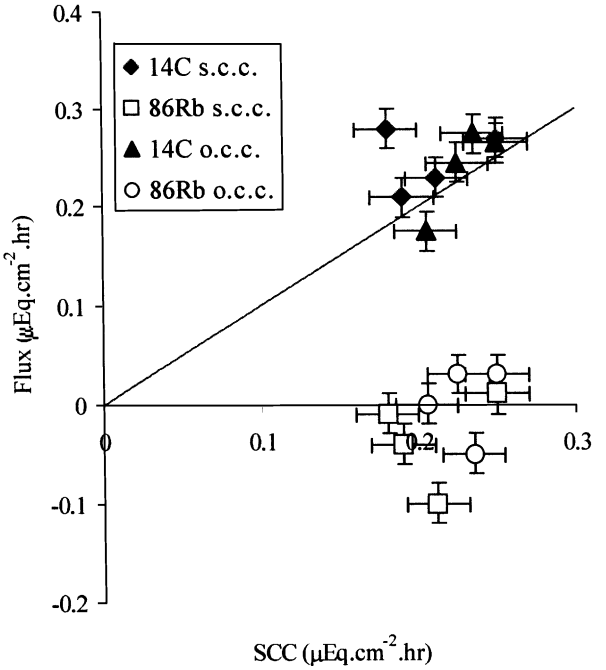

Fig. 2. The ^{14}C and ^{86}Rb flux of the rabbit corneal endothelium as a function of s.c.c. at 22 ± 0.5 °C. Short-circuit current (s.c.c.) ^{14}C (*filled squares*), open-circuit-current (o.c.c.) ^{14}C (*filled triangle*), s.c.c. ^{86}Rb (*empty squares*), and o.c.c. ^{86}Rb (*empty circles*) flux data of the rabbit corneal endothelium as a function of s.c.c. at 22 ± 0.5 °C ($n = 8$). The line indicates where the flux is equal to the s.c.c.

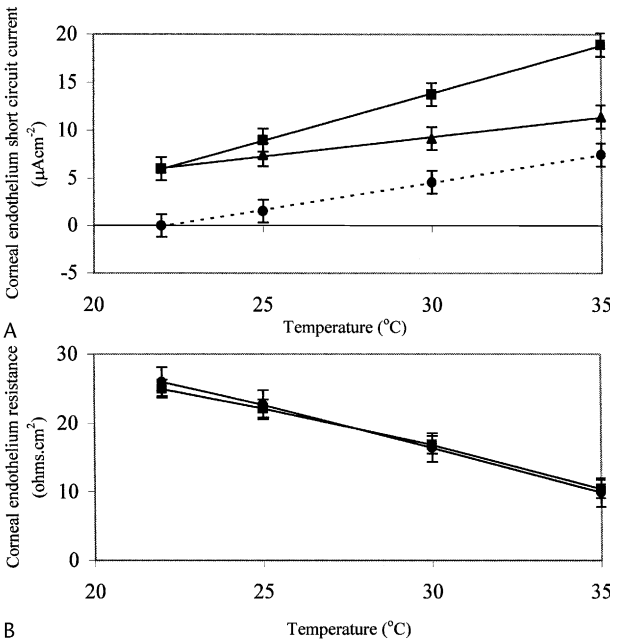

Fig. 3. (*A*) Amiloride inhibition of the rabbit corneal endothelium short-circuit current (s.c.c.) at 35.0, 30.0 and 25.0 ± 0.5 °C. *Filled squares* show total s.c.c., *filled triangles* show the amount of the s.c.c. remaining after perfusion of the sodium channel blocker amiloride (0.5 mM), *filled circles* show the amount of the s.c.c. inhibited by amiloride (i.e., generated by the NHE). (*B*) Endothelial resistance (R_e) with (*filled squares*) and without (*filled circles*) amiloride at 35.0, 30.0 and 25.0 ± 0.5 °C.

circuit compared to short circuit and that the net flux for potassium/rubidium would be expected to change by about 2%. Because our experimental uncertainty in these studies was about 7%, our technique was too insensitive to detect these expected minor changes in the net fluxes of bicarbonate and rubidium between our open-circuit and closed-circuit preparations.

The net fluxes were unambiguous: there is no significant rubidium flux across the rabbit corneal endothelium at 22 °C and the net bicarbonate flux equalled the short-circuit current within the limits of the experimental error of ± 7%.

The two types of data, net osmotic fluxes and electrical, show that the presence of bicarbonate is necessary for the whole transport process and is also of sufficient magnitude to account for the magnitude of the transport process. Necessity and sufficiency are persuasive indicators, which suggest that at 22 °C a bicarbonate ion current alone passing through the endothelial cells is responsible for maintaining corneal hydration.

The mechanism of the action is likely to be as follows. Corneal endothelial cell plasma membrane when isolated and formed into vesicles exhibits an active sodium/bicarbonate cotransporter activity (NBC) that operates in the direction outside to inside the cell (Lane et al., 2000). The NBC activity has been localized to the baso-lateral membranes of the cell (Sun et al., 2000).

Also located on the basolateral membranes of these cells is the Na^+-K^+-ATPase activity (Wigham,

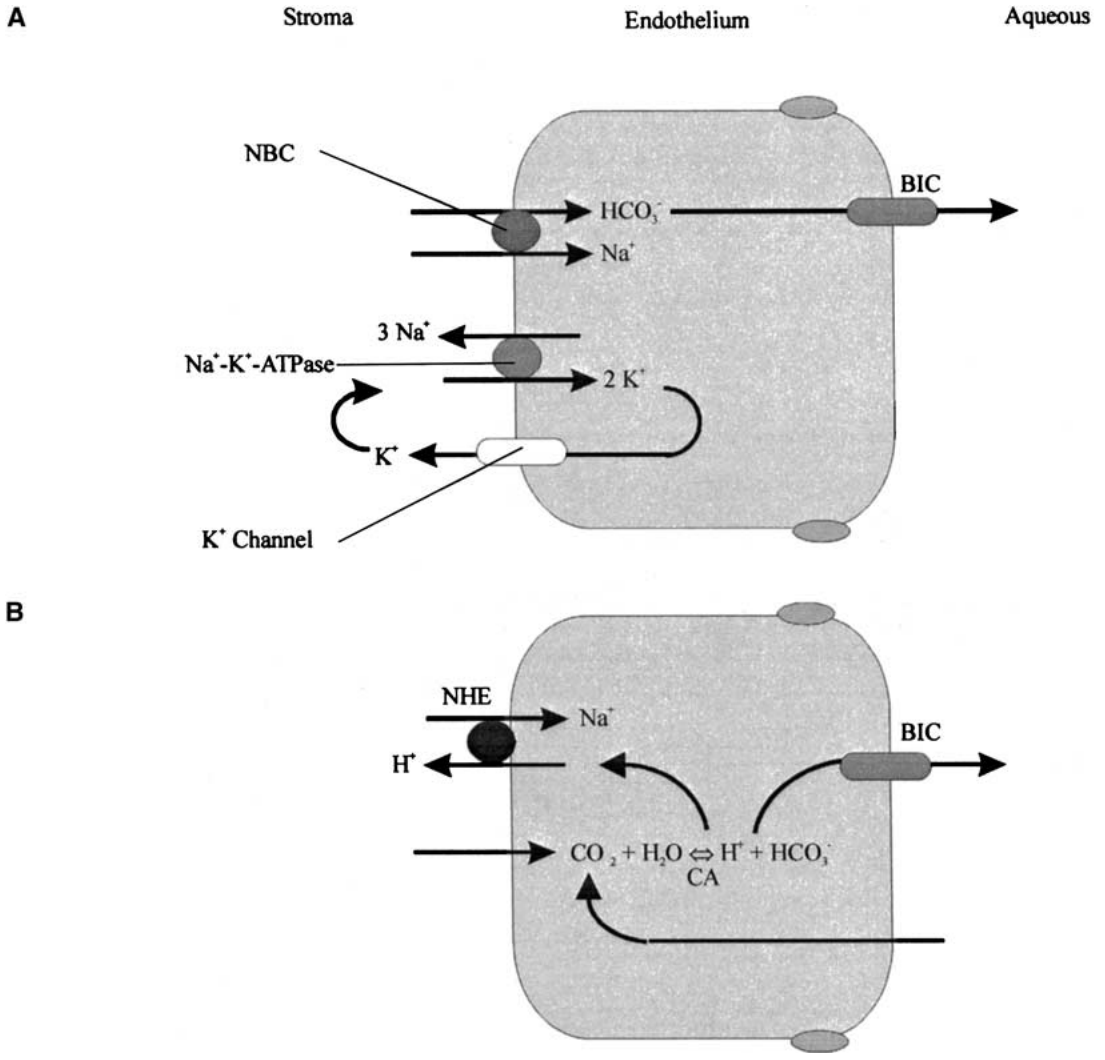

Fig. 4. (*A*) Proposed model for corneal endothelial ion transport mechanism operating at 22 °C. *See* Discussion for explanation. NBC, sodium bicarbonate co-transporter; BIC, bicarbonate ion channel. (*B*) Proposed model for additional ion transport mechanism operating at 35 °C. *See* Discussion for explanation.

Guggenheim & Hodson, 1994). There is no net sodium flux across the short-circuited corneal endothelium (Wigham & Hodson, 1985), which is consistent with the sodium being pumped out of the cell into the stroma and re-entering the cell through the basolateral NBC, thereby increasing intracellular bicarbonate above its electrochemical potential, which may contribute to increase the intracellular pH (Che et al., 1992). The 'high' bicarbonate then exits through an anion channel in the apical membrane, which is spontaneously active at physiological pH (Jones et al., 1992). The bicarbonate 'pump' operating at 22°C is illustrated in Fig. 4*A*. The present data, which show no net potassium/rubidium flux, indicate that potassium exits the cell across the basolateral membrane.

At 22°C, the bicarbonate pump of the corneal endothelium presents as a fairly straightforward operation. We suggest that at 35°C, a second major pathway for sodium re-entry across the endothelial baso-lateral membrane comes into play, which is illustrated in Fig. 4*B*: the amiloride-inhibitable Na/H exchanger (NHE). This transport activity is inactive at 22°C but is active at 35°C. Although this activity has not been localized in the corneal endothelium, because of the arguments given above relating to the absence of a net sodium flux across short-circuited endothelium, it can be reasonably suggested to be localized in the baso-lateral membranes. This second pathway is illustrated in Fig. 4*B*. It is not operational at 22°C, but at 35°C it may be seen as operating in parallel with the bicarbonate pump pathway illustrated in Fig. 4*A*. Although it is not obvious how this pathway, acting through a neutral exchanger such as NHE, could contribute to an electrogenic transport system, we propose that it could be possible. As in

Fig. 4*A*, the sodium enters and exits the cell on the same basolateral face (exit pathway via Na-K-ATPase, not shown in Fig. 4*B*) and so can play no role in any trans-endothelial electrogenic transport. In the proposed model, CO_2 and water dissociate into bicarbonate ions and protons, which exit the cell via opposite faces. This pathway releases positively charged hydrogen ions into the tear-side extracellular space of the endothelium and negatively charged bicarbonate ions into the lens-side compartment. This proposed pathway is therefore electrogenic overall and results in the electrical equivalent of a negative current flow across the endothelium into the lens side. At the same time, the transport pathway could be osmotically neutral, because it deposits osmotica at equal rates on either side of the monolayer: hydrogen ions on the tear-side, bicarbonate ions on the lens-side. This suggests that hydrogen ions exhibit an osmotic pressure and although this has not been demonstrated experimentally, there seems no obvious reason why this should not be so. However, in vivo, both these fluxes would be quickly neutralized and recombined through the paracellular route. This model pathway is consistent with the observations that inhibition either of NHE activity or of carbonic anhydrase inhibition decreases the short-circuit current across the endothelium without affecting the water relations of the preparation (Wigham et al., 1996). It can also explain in a semi-quantitative fashion the discrepancy between measured net bicarbonate flux and s.c.c. at 35 °C, which provided the original stimulus for this study, by taking into account the incomplete labelling of the bicarbonate in the pathway shown in Fig. 4*B* because CO_2 may enter the cell from either face. The purpose of the second pathway could be to regulate intracellular pH. At 35 °C, corneal endothelium exhibits a mandatory aerobic glycolysis (Riley & Winkler, 1990) releasing protons into the cell, and so the purpose of the second pathway could be to drive the pH more basic and so prevent aerobic glycolysis from interfering with the pump pathway shown in Fig. 4*A*.

This study was initiated in the Department of Optometry and Vision Sciences, Cardiff University, Wales.

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