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Two pathways for electrogenic bicarbonate ion movement across the rabbit corneal endothelium

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Abstract

Amiloride (0.5 mM) inhibited the rate of entry of Na^+ into corneal endothelial cells by more than half ((0.76 ± 0.10) to (0.21 ± 0.10) μEq cm^{-2} h^{-1}). The same concentration of amiloride caused only minimal disturbance to corneal hydration control by the endothelium (range 0–12%). Amiloride (0.5 mM) and acetazolamide (1 mM) reversibly inhibited trans-endothelial short circuit current by about a half. Their combined effect was not additive. Acetazolamide (1 mM) reduced net HCO_3^- flux across the short-circuited endothelium by about the same amount ((0.50 ± 0.11) μEq cm^{-2} h^{-1}) that amiloride (0.5 mM) reduced Na^+ entry into the cells ((0.55 ± 0.14) μEq cm^{-2} h^{-1}). Low concentrations of amiloride (10 μM) had little effect on the transport characteristics of the endothelium, indicating that Na^+ entry into the endothelial cells under physiological conditions is not primarily through Na^+ channels. The data are consistent with an Na^+/H^+ exchanger acting in tandem with carbonic anhydrase through a pathway which could have a regulatory role on endothelial transport via its effect on Na^+ re-entry. Residual trans-endothelial HCO_3^- transport, apparently unaffected by amiloride or acetazolamide inhibition, is calculated to be of sufficient magnitude to maintain corneal hydration.

Keywords: Cornea; Endothelium; Bicarbonate; Amiloride; Acetazolamide; Fluid flow

1. Introduction

The osmotically active 'pump' in mammalian corneal endothelium, which regulates corneal hydration and consequently corneal transparency [1], has been identified as a metabolically coupled flux of HCO_3^- ions that flows in the direction from corneal stroma to aqueous humour [2–4]. Experimental evidence has been used to model the membrane events which drive the HCO_3^- ion current [2,5–7]. There are, however, significant differences between these models and so there is a need to discriminate between some of the options. When HCO_3^- is moved across the endothelial cell it requires an input of metabolic energy [3,4] and there is a consensus that this energy is provided by plasma-membrane-bound Na^+/K^+-ATPase activity coupled to the HCO_3^- movement [3,8,9]. As HCO_3^- is a net transported ion, then either its movement from corneal stroma into cytoplasm across the basolateral membrane or its exit from the cytoplasm into the aqueous across the apical cell membrane must be driven uphill. Localisation of the Na^+/K^+-ATPase to the basolateral membrane taken together with the absence of a net Na^+ flux in the short-circuited preparation ([10,11]; in contrast to the net Na^+ flux observed in open circuit preparations, [12,13]) suggests that the driven step (coupled to the trans-membrane Na^+ gradient) takes place at the basolateral membrane [14]. This view is also supported by the relatively high pH measured in the cytoplasm, indicating that HCO_3^- entry into the cell is uphill [15,16]. The various models suggest a number of possible mechanisms for coupling the trans-membrane Na^+ gradient to HCO_3^- movement and this coupling has been proposed to be direct, via an Na^+/HCO_3^- co-transporter [7,17], or indirect, utilising an $Na^+/K^+/2Cl^-$ co-transporter coupled to a Cl^-/HCO_3^- exchanger [18]. Other models suggest that an Na^+/H^+ exchanger located at the basolateral membrane can contribute to the generation of electrogenic HCO_3^- movement [5,19]. The possible involvement of the Na^+-dependent HCO_3^-/Cl^- exchanger or the recently described K^+/HCO_3^- cotransport mechanism have not yet been seriously considered for the corneal endothelium [20,21]. In this study we carried out a series of experiments to

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determine whether Na^+/H^+ exchanger activity can contribute to the trans-endothelial electrogenic HCO_3^- flux. We conclude that, although most Na^+ enters the cell via the Na^+/H^+ exchanger and that this activity contributes to net electrogenic HCO_3^- transfer into the aqueous humour, in addition to this activity there must be another electrogenic HCO_3^- transfer mechanism which is primarily responsible for net osmotic transfer across the endothelium.

2. Methods and materials

Animals used in all of these experiments were cared for using the Code of Practice for the Housing and Care of Animals used in Scientific Procedures (1989; HC 107). New Zealand White or Dutch rabbits were killed by an overdose of sodium pentobarbital administered via the marginal ear vein. After death, the eyes were enucleated and used immediately or refrigerated at 4°C. All eyes were used within 12 h post mortem.

2.1. Membrane potential (PD_m) and intracellular Na^+ concentration ($[Na^+]_i$)

De-epithelialised corneas were mounted in a Perspex chamber that allowed the cells to be impaled with microelectrodes. Preparations were perfused at 10 ml h^{-1} with Ringer containing (mM): NaCl, 113; KCl, 4.6; $NaHCO_3$, 21; $MgSO_4$, 0.6; glucose, 7.5; reduced glutathione, 1.0; Na_2HPO_4, 1.0; Hepes, 10; $CaCl_2$, 1.4; bubbled with 5% CO_2, 7% O_2 in N_2 and pH set at 7.4. A water jacket maintained the preparation at 35°C.

Filamented borosilicate glass capillaries (1 mm o.d.) were pulled into microcapillaries, which when filled with 154 mM KCl for measurement of PD_m had tip resistances of typically 100–200 MΩ. Before use as ion-selective microelectrodes, the glass microcapillaries were baked at 200°C in air to dry the glass and then in tributylchlorosilane vapour to prepare the surface for the Na^+ ionophore (Fluka). After filling with ionophore the microcapillaries were left overnight with their tips immersed in 154 mM NaCl, backfilling the next day with 154 mM KCl completed electrode preparation. Detailed procedures for preparation, filling, calibration, mounting and impalement of endothelial cells with potential and ion-sensitive microelectrodes have been given previously [14,22].

To determine the effect of amiloride on Na^+ re-entry into the cell, measurements of PD_m and sodium potential (PD_{Na}) were made by impaling the cells with reference and Na^+ selective microelectrodes. Whilst the cells were impaled the preparation was perfused firstly with Ringer plus amiloride (0.5 mM; the small chamber volume enabled the solution change to be made in approximately 2 min) and then with Ringer containing amiloride (0.5 mM) plus ouabain (0.5 mM). The rate of change of $[Na^+]_i$ calculated from the measurements of PD_m and PD_{Na} following ouabain inhibition is dependent on the passive permeability pathways for Na^+ re-entry into the cell.

2.2. Specular microscopy

De-epithelialised rabbit corneas were mounted under the specular microscope using the method of Dikstein and Maurice [1]. The epithelial surface of the cornea was sealed under silicone oil and the endothelial surface perfused at 50 ml h^{-1} with Ringer warmed to 35°C. Perfusion pressure was set at 15 cm H_2O to maintain corneal geometry. After mounting, the preparation was allowed to stabilise its thickness, which was achieved within 1 h. To determine the effect of amiloride on the ability of the endothelium to maintain corneal hydration, the preparation was stabilised in Ringer for 1 h. followed by Ringer plus amiloride (0.5 mM) for 1.5 h, followed by 1 h of Ringer plus amiloride (0.5 mM) plus ouabain (0.5 mM). Five readings of corneal thickness were taken at 10 min intervals for the duration of the experiment. In half of the preparations ($n = 5$), the de-epithelialised surface was exposed to Ringer solution to induce stromal swelling. When the preparation had swollen by approximately 200 μm, the Ringer was replaced with silicone oil. In this way it was possible to monitor the effect of 0.5 mM amiloride on the ability of the endothelium to de-swell a hydrated corneal stroma.

2.3. Trans-endothelial potential difference (PD_e), resistance (R_e), short circuit current (s.c.c.) and net HCO_3^- and Na^+ flux

De-epithelialised corneas were mounted between the two halves of a modified Ussing type chamber and perfused at 10 ml h^{-1} with Ringer solution. The whole apparatus was maintained at 35°C by immersion in a thermostatically controlled oil bath. Potential difference was measured continuously and resistance of the preparation (series resistance + endothelial resistance; R_t) measured at intervals. Transendothelial resistance (R_e) was calculated by subtraction of the series resistance (R_s; measured at the end of the experiment when the endothelium had been removed) from R_t. A more detailed description of the apparatus and its use has been given previously [23]. Inhibitors were dissolved in Rabbit Ringer and perfused through both half chambers. Perfusion was continued until any changes in PD_e and R_e were stable. R_e has been shown to be inversely proportional to endothelial cell permeability [24] and any significant change in R_e was considered to be a toxic effect when such changes were irreversible. When R_e is unchanged, s.c.c. (related to HCO_3^- pump activity; [10]) is a monitor of endothelial HCO_3^- transport activity. The effects of amiloride or acetazolamide and the combinations of the two were investigated. To measure net HCO_3^- and Na^+ fluxes, unidirec-

tional fluxes were measured by loading one half chamber with radio-labelled HCO_3^- and Na^+ and collecting samples from both sides, followed by a reversal of loading. The preparation was short-circuited whilst the unidirectional fluxes were measured. A detailed description of the short-circuiting technique and measurement of unidirectional ion fluxes is given elsewhere [10,24]. The effect of 1 mM acetazolamide on unidirectional fluxes was measured. The requirement of several hours of stability for flux investigations on these preparations debarred the use of amiloride.

3. Results

In a series of preliminary experiments amiloride (0.5 mM) was found to hyperpolarise PD_m from -29.5 ± 5.7 to -41.0 ± 4.0 mV (mean ± S.E., $n = 13$) over a period of about 20 min. In these experiments, where cells were impaled with PD_m- and Na^+-selective microelectrodes simultaneously, amiloride (0.5 mM) induced a hyperpolarisation of both PD_m and the potential detected with the sodium-selective microelectrode (PD_{Na}). Intracellular Na^+ concentration ($[Na^+]_i$) calculated from these values decreased significantly from (12.2 ± 1.6) to (7.7 ± 1.5) mM (mean ± S.E., $n = 9$, $P < 0.05$) after exposure to 0.5 mM amiloride. When the cells were perfused with amiloride + ouabain, both PD_m and PD_{Na} depolarised. Fig. 1 shows a typical trace of one such experiment. The pattern of depolarisation was reproducible and from it the initial rate of influx of Na^+ (across the baso-lateral membrane) in the presence of amiloride (plus ouabain) was calculated to be 0.21 ± 0.04 μEq cm^{-2} h^{-1} (mean ± S.E., $n = 7$). This

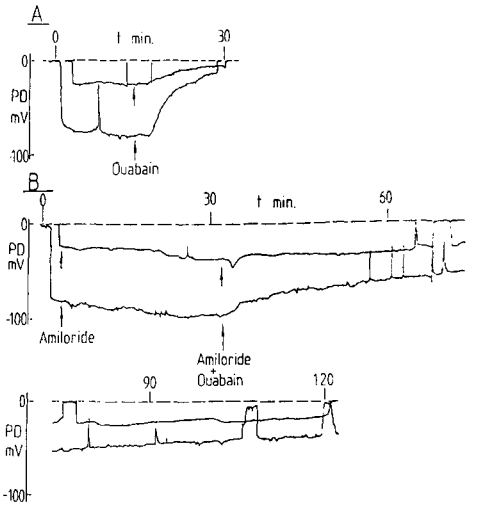

Fig. 1. A typical trace of rabbit corneal endothelial cell PD_{Na} (lower line) and PD_m (upper line). Trace A shows the depolarising effect of ouabain (0.5 mM). Trace B shows the hyperpolarising effect of amiloride (0.5 mM) and the change in the pattern of depolarisation when amiloride + ouabain (0.5 mM) was added. Amiloride is seen to slow considerably the ouabain-induced depolarisation.

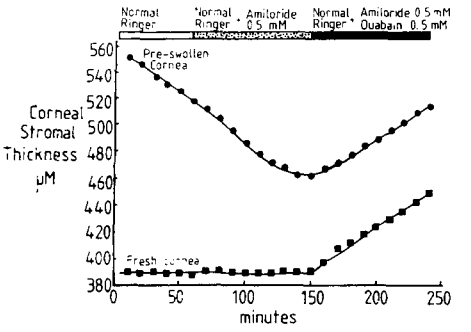

Fig. 2. A typical trace of experiment to determine the effect of amiloride (0.5 mM) on the ability of rabbit corneal endothelium to maintain corneal thickness. The lower trace shows a normal cornea, the upper trace shows a cornea that has been allowed to swell prior to the start of the experiment. The amiloride is seen to have little or no effect on maintenance of hydration or deturgescence from the swollen state. The addition of ouabain (0.5 mM) showed the preparation to be responsive to inhibition.

was significantly less than the uninhibited influx of Na^+ (with ouabain alone) which was 0.76 ± 0.05 μEq cm^{-2} h^{-1} (mean ± S.E., $n = 4$, $P < 0.05$). These observations indicate the presence, in the basolateral membrane of these cells, of an amiloride-inhibitable traffic protein which is responsible for a major part of the re-entry of Na^+ into fresh corneal endothelial cells. The elimination of Na^+ re-entry via the Na^+/H^+ exchanger could explain the reduction in $[Na^+]_i$ after amiloride inhibition, whilst the removal of this permeability pathway for Na^+ across the membrane might explain the observed hyperpolarisation of the membrane after amiloride inhibition (towards the K^+ equilibrium potential).

The ability of the endothelium to maintain corneal thickness and hydration and the effect of amiloride is shown by the traces in Fig. 2. Amiloride (0.5 mM) had no effect on the ability of the endothelium to maintain physiological thickness, nor did it affect its ability to thin a pre-swollen cornea. Pre-swollen corneas thinned at (36.0 ± 3.0) μm h^{-1} (mean ± S.E., $n = 5$) in the absence or presence of amiloride. Addition of ouabain showed the amiloride exposed preparation still to be responsive to transport inhibition.

At 0.5 mM, amiloride reduced s.c.c. from (37.9 ± 2.5) to (18.7 ± 1.8) μA cm^{-2}, an inhibition of (51.3 ± 2.4)% and caused no significant change in R_e, (15.0 ± 0.9) to (14.1 ± 0.8) Ω cm^2 (mean ± S.E., $n = 11$). At or below 0.5 mM, the amiloride effects were fully reversible. A typical trace showing the inhibition and reversibility of effect of 0.5 mM amiloride is shown in Fig. 3. At concentrations of amiloride greater than 0.5 mM, R_e was irreversibly reduced, indicating a possible toxic or non-specific effect of the drug on the endothelial cells. The effect of 1.0 mM acetazolamide was similar to the effect of 0.5 mM amiloride; at 1.0 mM acetazolamide reduced s.c.c. from (37.1 ± 1.8) to (20.8 ± 4.0) μA cm^{-2}, an inhibition of (37.1 ± 5.0)%, R_e was unchanged (18.3 ± 3.0) to $(18.2 \pm$

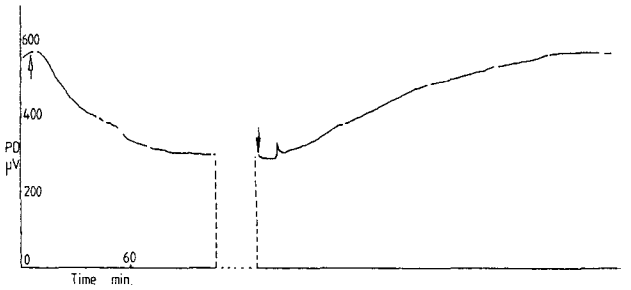

Fig. 3. A typical trace of the effect of amiloride (0.5 mM) on trans-endothelial potential difference. Amiloride was added at the open arrow, normal Ringer was perfused at the closed arrow. The dashed area on the time axis represents a period of 115 min during which time PD_e was stable. The amiloride effect was completely reversible. Half-exchange time for the chamber is approx. 5 min. Gaps in the trace indicate points at which R_e was measured, it remained unchanged throughout the experiment.

3.0) Ω cm^2 (mean ± S.E., $n = 4$). At concentrations above 1.0 mM there was a small change in R_e. At or below 1 mM all effects were fully reversible. A typical trace showing the effect of the combined addition of amiloride (0.5 mM) and acetazolamide (1.0 mM) on s.c.c. is shown in Fig. 4. The combination of inhibitors reduced s.c.c. by (62.7 ± 1.9)% (mean ± S.E., $n = 6$). Whilst this reduction was significantly greater than the single inhibitor effect, $P < 0.05$, Student's t-test, it was very much less than the inhibition expected (88.4%) if the inhibitors were acting independently and additively. The order of presentation of the drugs was not important; the combination of inhibitors decreased s.c.c. to (60.3 ± 2.5)% when amiloride was presented first and (65.0 ± 2.4)% when acetazolamide was presented first (mean ± S.E., $n = 3$).

The apparent contradiction in the above data; that inhibitors which reduce trans-endothelial s.c.c. (considered to be representative of endothelial pump activity, [10]) and Na^+ exchange rates across the basolateral membrane have little or no effect on the ability of the endothelium to maintain corneal stromal hydration, led us to repeat published reports of inhibitor effect on ion fluxes across the preparation. Amiloride-inhibited preparations were not sufficiently stable for a long enough time period to make measurements of trans-endothelial net ion fluxes. However, acetazolamide (1 mM) reduced net HCO_3^- flux, under short-circuited conditions, from (1.20 ± 0.10) to (0.69 ± 0.06) μEq cm^{-2} h^{-1} (mean ± S.E., $n = 3$), i.e., by 42.7%. In these experiments s.c.c. was reduced from (1.76 ± 0.2) to (1.05 ± 0.1) μEq cm^{-2} h^{-1}. In neither the control nor the acetazolamide-inhibited preparation was there a significant net Na^+ flux across the short-circuited endothelium, Fig. 5.

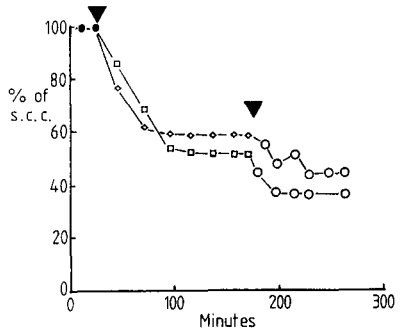

Fig. 4. Typical traces to show the inhibitory effects of amiloride (0.5 mM), □——□; acetazolamide (1 mM), ◇——◇; and both, ○——○, on rabbit corneal endothelial cell trans-endothelial s.c.c.

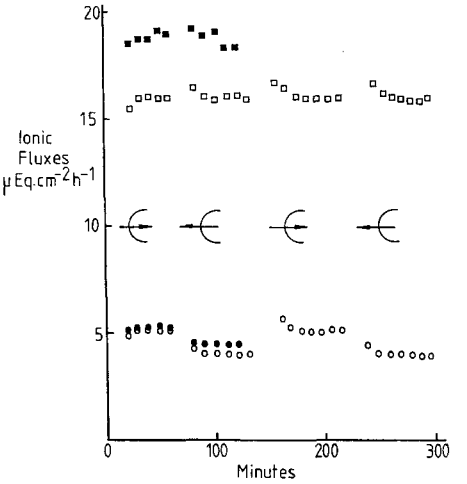

Fig. 5. Unidirectional ion fluxes across corneal endothelial cells mounted in vitro. Open circles indicate HCO_3^- fluxes across a control cornea, filled circles are from a cornea exposed to 1 mM acetazolamide. Open squares indicate Na^+ fluxes across a control cornea, filled squares are from a cornea exposed to 1 mM acetazolamide.

4. Discussion

Amiloride (0.5 mM) reduced the rate of Na^+ entry into corneal endothelial cells by more than half. Amiloride (0.5 mM) and acetazolamide (1 mM) each reversibly inhibited trans-endothelial s.c.c. by about half and at these concentrations neither drug significantly affected (< 5%) the passive permeability of the endothelial monolayer. When added simultaneously to the endothelium, if their individual effects had been additive, an inhibition of 88% would be expected. The observed inhibition of only 62% suggests that the drugs could be acting on the same pathway [5]. Preliminary data on the dose–response curve for both amiloride and acetazolamide suggested that around 60% of s.c.c. is likely to be the maximum inhibition with either drug. However, the concentrations that elicited 60% inhibition (1.0 mM amiloride and 5 mM acetazolamide) caused irreversible permeability increases of (19.6 ± 6.7)% for amiloride and (14.4 ± 2.4)% (mean ± S.E., $n = 3$) for acetazolamide. These irreversible changes associated with the higher and probably saturating doses of acetazolamide or amiloride were too unstable to allow systematic investigations of the transport properties of the endothelium and

were consequently not pursued. The concentrations used in these experiments (0.5 mM amiloride and 1 mM acetazolamide) we estimate, gave 80% saturation. Even at the lower concentrations of amiloride (0.5 mM) it was not possible to maintain stable preparations for the several hours required to carry out unidirectional isotopic flux studies.

It was of interest to note that the reduction in net HCO_3^- flux across the preparation after inhibition with 1 mM acetazolamide was about the same as reported previously [25] and was about the same as the amiloride induced reduction in Na^+ flux into the cell. Net fluxes of Na^+ were, as reported before [10], not observed across the short circuited endothelium (in contrast to the well established open circuit net Na^+ flux). These data tend to confirm the proposal that a circulating basolateral Na^+ flux into and out of the cell drives the net HCO_3^- flux across the cell. The stoichiometry suggests that for each Na^+ driven into the cell across the basolateral membrane, one HCO_3^- exits across the apical surface of the cell via the acetazolamide/amiloride inhibitable pathway. Because low doses of amiloride, capable of blocking Na^+ channels had no effect on the endothelial transport characteristics, we conclude that Na^+ entry into the cell via this pathway is likely to be via the Na^+/H^+ exchanger whose presence has been proposed from electrophysiological studies [26] and from studies on vesicles prepared from purified corneal endothelial cell plasma membranes [27]. We propose a model that describes HCO_3^- exit from corneal endothelial cells by the pathway illustrated in Fig. 6.

There seems to be relatively little osmotic activity associated with this pathway. In seven of our corneal preparations the exposure of the endothelium to 0.5 mM amiloride had no significant effect ($< 10\%$) on either corneal thickness in stabilised preparations ($n = 4$) or on deturgescence rates of pre-swollen corneas ($n = 3$) There was a marginal effect on the other five preparations, but

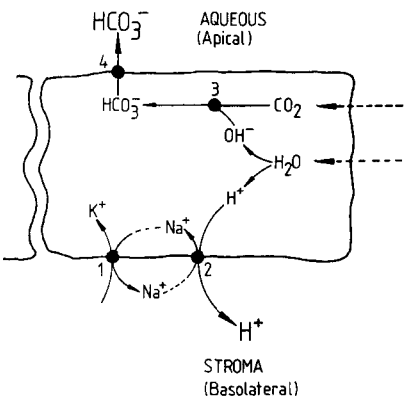

Fig. 6. A model of that proportion of endothelial HCO_3^- transport that is amiloride and acetazolamide inhibitable. Components are numbered: 1, Na^+/K^+-ATPase; 2, Na^+/H^+ exchanger; 3, carbonic anhydrase; 4, unidentified anion transporter. This HCO_3^- transport system is electrogenic but shows little osmotic activity.

always less than 20% inhibition (ouabain-inhibited preparations were taken to show 100% inhibition of trans-endothelial osmotic transfer). A similar range of results has been reported for acetazolamide inhibition of osmotic transfer, varying between 0% inhibition up to 25% inhibition [28,29]. It is noticeable that osmotic transfer inhibition by acetazolamide seems to increase progressively with time, perhaps reflecting not only osmotic transfer characteristics but also possibly diminishing endothelial barrier characteristics. Kuang et al. [28] showed little or no immediate effect on fluid transfer of 5 mM acetazolamide but a progressive decrease over a 4 h period. The progressive change of fluid transfer characteristics is close to the progressive increase in endothelial permeability which we have noted during the course of this study. Carbonic anhydrase inhibition with other drugs such as ethoxyzolamide, also shows variations in osmotic transfer inhibition. However, inspection of the primary data suggests that the magnitude of the inhibition increases progressively with time. In all cases, in the first hour of study it appears that no-one has reported an apparent osmotic transfer inhibition greater than about 25% [5,29,30]. The published reports of amiloride inhibition of osmotic transfer have used 1 mM amiloride [31] which concentration we found to be associated with secondary effects on the tissue. In this study we find a 0–15% inhibition of osmotic transfer in the presence of amiloride under the same incubation conditions that produces a 42% reduction in net HCO_3^- transfer and a greater than 50% inhibition of Na^+ entry into the cells.

Whilst the pathway is seen from the s.c.c. measurements to be electrogenic and the drugs act quickly in reducing s.c.c., the osmotic transfer data suggest that there is relatively little associated osmotic activity. In the model shown in Fig. 6, HCO_3^- and H^+ are produced inside the cell by the carbonic anhydrase catalysed reaction between H_2O and CO_2. The HCO_3^- produced exits the cell across the apical membrane via a traffic protein yet to be determined and it is this component of the total HCO_3^- net flux that is eliminated by inhibition with acetazolamide. The H^+ produced exits the cell via the Na^+/H^+ exchanger located on the basolateral membrane, which is driven by the energy of Na^+ re-entering the cell down its electrochemical gradient after being driven out by Na^+/K^+-ATPase also located on the basolateral membrane. At steady state, Na^+ recycles across the basolateral membrane with exit flux via Na^+/K^+-ATPase equal to its entry flux via the Na^+/H^+ exchanger and, it has been suggested, by at least one other route [27]. The consequence is that passive Na^+ entry via the Na^+/H^+ exchanger is non-electrogenic: the Na^+ is simply recycling across the basolateral membrane. H^+ exit via the exchanger represents a net movement of positive charge into Descemets membrane. The electrogenic effect of the flux of H^+ into Descemets and the flux of negative ions (HCO_3^-) into the aqueous are, electrically, additive. In conclusion we can

see that the pathway illustrated in Fig. 6 is electrogenic, with a movement of opposite charges in opposite directions across the apical and basolateral membranes, which manifests itself as a component of the s.c.c. across the preparation.

In order to generate and maintain an osmotic gradient across an epithelium, clearly it would be neccessary to effect a net osmotic transfer across the epithelium in open circuit (although it must be acknowledged that there is, as yet, no generally agreed model to explain local osmotic coupling). There is a method by which the metabolically driven movements of H^+ and HCO_3^- out of opposite faces of the corneal endothelium could generate, in open circuit, an osmotic gradient across the monolayer. The method would require the paracellular route to be permselective. Experimentally, the endothelium shows a very slight cation selectivity [24], but for practical purposes, the monolayer is effectively non-selective and is therefore unlikely to produce any significant osmotic gradient across the endothelium in open circuit from the pathway shown in Fig. 6. Whilst the acetazolamide/amiloride-inhibitable pathway of HCO_3^- transport across corneal endothelium proposed here probably places a considerable demand on the metabolism of the cells, we do not believe that it is primarily responsible for the regulation of corneal hydration by osmotic transfer.

It seems clear that the amiloride/acetazolamide-inhibitable pathway illustrated in Fig. 6 does not contribute 100% of the HCO_3^--dependent transport activity across the corneal endothelium and nor is it primarily responsible for maintaining corneal hydration. The question arises: does the other HCO_3^--dependent pathway, not yet characterised here but variously proposed to involve Na^+/HCO_3^- re-entry or $Na^+/K^+/Cl^-$ re-entry of Na^+ into the cell, have sufficient activity to maintain hydration? In examining this quantitative question we used the mathematical description of the endothelial pump/leak mechanism originally derived by Hedbys and Mishima [32] and independently derived by Hodson [33] which has the equation:

$$J_p = \frac{\omega}{\sigma}\Delta\gamma$$

where J_p is the osmotically active HCO_3^- pump, σ is the endothelial reflection coefficient to HCO_3^-, ω is the endothelial permeability to HCO_3^- and $\Delta\gamma$ is nowadays given as corneal gel pressure [34].

Independent measurements of σ, ω and $\Delta\gamma$ may be combined to predict the magnitude of the pump, J_p, needed to sustain corneal hydration. In order to derive a semi-quantitative estimate, we used the values of these parameters previously published by this laboratory: $\sigma = 1$ [35]; $\omega = 0.214$ cm h^{-1} [24]; $\Delta\gamma = 2.6$ mM [34]; and give a predicted value of J_p of 0.55 μEq HCO_3^- cm^{-2} h^{-1}. It seems that from the acetazolamide-inhibited preparations, where the residual HCO_3^- net flux is about 0.6 μEq HCO_3^- cm^{-2} h^{-1}, that there is likely to be sufficient activity to maintain corneal hydration. It is interesting to note that the residual Na^+ influx after amiloride inhibition suggests a 1 Na^+ (influx): 2 HCO_3^- (efflux) stoichiometry. This ratio is consistent with the model of direct basolateral coupling via a $1\,Na^+/2\,HCO_3^-$ symport [2,6]. So far, we have been unable to detect any such direct HCO_3^-- or HCO_3^--gradient-dependent Na^+ influx in experiments with plasma membrane vesicles isolated from corneal endothelial cells [27].

We provide evidence in support of the existence of an electrogenic HCO_3^- pathway in corneal endothelium including a baso-lateral Na^+/H^+ exchanger and cytoplasmic carbonic anhydrase. As the pathway is only marginally involved in osmotic transfer across the corneal endothelium we suggest that it probably has a regulatory role which could involve pH regulation or might provide a mechanism for Na^+ reentry into the cell that dissipates the Na^+ gradient across the basolateral membrane and reduces the ion gradient that is driving the osmotic pump re-entry mechanism. However, we believe from unpublished evidence from this laboratory that the pathway we describe here may be involved in regulating the permeability of the endothelial monolayer.

Acknowledgements

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