Published in final edited form as:

Exp Eye Res. 2012 February ; 95(1): 2–7. doi:10.1016/j.exer.2011.06.004.

“Molecular Mechanisms Underlying the Corneal Endothelial Pump”

Joseph A. Bonanno

Indiana University, School of Optometry, Bloomington, Indiana 47405

Abstract

The corneal endothelium is responsible for maintaining the hydration of the cornea. This is through a “Pump-Leak” mechanism where the active transport properties of the endothelium represent the “Pump” and the stromal swelling pressure represents the “Leak”. For the “Pump”, Na^+ , K^+ ATPase activity and the presence of HCO_3^- , Cl^- , and carbonic anhydrase activity are required. Several basolateral (stromal side) anion transporters, apical (facing the aqueous humor) ion channels and water channels have been identified that could support a model for ion secretion as the basis for the endothelial pump, however evidence of sustained anion fluxes, osmotic gradients or the need for water channels is lacking. This has prompted consideration of other models, such as Electro-osmosis, and consideration of metabolite flux as components of the endothelial pump. Although the conditions under which the “Pump” is supported are known, a complete model of the endothelial “Pump” has yet to emerge.

Keywords

corneal endothelium; transparency; ion & fluid transport

1. Introduction

The topic of molecular mechanisms underlying the corneal endothelial pump was extensively reviewed in 2003 (Bonanno 2003). The purpose here is to summarize the important features of the endothelial pump and provide an update on recent significant findings. Development of new techniques, e.g. siRNA knockdown and *in vivo* viral transfection of shRNA, has produced more specific testing of pump features. Similarly, the use of knockout models (e.g., AQP1) has prompted significant re-thinking of the nature of solute-fluid coupling. These molecular approaches together with new studies using customary pharmacological approaches for studying corneal endothelial fluid transport have led to re-evaluations of the corneal endothelial pump.

2. Background

The cornea is the major refractive element of the eye. This requires optical transparency and smooth curved surfaces. The cornea has five layers: the outer epithelium, Bowman’s (basement) membrane, the stroma, Descemet’s (basement) membrane, and the inner surface

© 2011 Elsevier Ltd. All rights reserved.

812-855-4440, jbonanno@indiana.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

endothelium. The smooth regular surface, tight packing of cells, relative paucity of organelles (especially mitochondria), and lack of blood vessels in the stratified squamous corneal epithelium (~50 μm) reduce light scatter thereby contributing to transparency. The corneal stroma reduces light scatter by its regular spacing among collagen fibers and uniform diameter of fibers. This contrasts with the sclera where spacing and fiber diameters have a very broad distribution. The regular spacing of the stromal fibers is controlled by the hydrophilic glycosaminoglycan (GAGs) ground substance. When the hydration of the stroma is ~3.5 mg $\text{H}_2\text{O}/\text{mg}$ dry tissue or less, the stroma is relatively transparent. A slit-lamp view of the stroma however, indicates that it scatters more light than the epithelium. This is due to the thin flat keratocytes that lie between stromal lamellae. Keratocytes are responsible for producing collagen and GAGs (among many other extracellular substances) and will be activated during trauma to repair the stroma. Repair alters the expression profile of keratocytes, which can lead to increased light scatter. In contrast, the corneal endothelium is a thin (4 μm) confluent monolayer that has a very high density of mitochondria, but because of its extremely short optical pathlength, scatters very little light.

The epithelium provides the barrier to the outside world, the stroma provides the refractive shape and the endothelium maintains the nutrition of the corneal cells and the hydration of the stroma.

Except for oxygen, all the nutrients for the cornea come from the aqueous humor and through the endothelium. For example, glucose transporters are present on both the apical (aqueous humor side) and basolateral (stromal side) endothelial cell membranes to allow transcellular glucose flux (Kumagai, Glasgow et al. 1994). Moreover, 85% of the glucose consumed by the cornea is converted to lactate, which diffuses posteriorly across the endothelium (Riley 1969). The corneal endothelium also maintains the hydration of the stroma through active transport mechanisms. Because of the swelling pressure (~60 mmHg) exerted by molecular repulsion from the highly negatively charged stromal GAGs, bare stroma can swell to many times its normal thickness (see (Kwok and Klyce 1990) for further details) and as a consequence the spacing between fibers becomes non-uniform, light scatter increases, and corneal transparency is lost. This tendency to swell is counteracted by the endothelial pump through the membrane active transport mechanisms. Steady-state hydration occurs when the endothelial pump rate equals the GAG driven leak. Maurice (Maurice 1981) called this the “Pump-Leak” mechanism for maintenance of corneal hydration and transparency. Because of the presence of the continuous leak, loss of endothelial ion transport activity leads to corneal edema, loss of transparency, and impaired vision.

3. Corneal Endothelial Pump Description

The endothelial pump function is best demonstrated using rabbit corneas mounted in modified Ussing chambers in vitro. If carefully mounted and perfused with appropriate media, the cornea will maintain its thickness for several hours. Another approach is to remove the epithelium, expose the anterior stroma for a short period to Ringer's solution allowing the stroma to swell, remove the anterior solution and replace it with silicone oil. The corneal thickness will then slowly decrease exponentially in a process called deturgescence. From these experiments it was determined that the cornea will swell, or not deturgesce, if the endothelium is exposed to the cardiac glycoside ouabain, an inhibitor of the Na^+, K^+ ATPase, indicating that the endothelial pump is dependent on primary active transport. Removal of HCO_3^- from the endothelial perfusing solution had a similar inhibitory effect on the pump and addition of carbonic anhydrase inhibitors slowed the pump by ~30%, indicating a major role for HCO_3^- (Dikstein and Maurice 1972; Fischbarg and Lim 1974; Hodson and Miller 1976; Riley, Winkler et al. 1995). Because Cl^- removal did not

cause initial swelling, the early pump model was described as an exclusive HCO_3^- secretory mechanism. Subsequent studies indicated that Cl^- was important for maintaining the pump (Winkler, Riley et al. 1992), suggesting that an anion exchanger, ($\text{Cl}^-/\text{HCO}_3^-$) played a role. Consistent with an anion transport mechanism, anion transport blockers, e.g. DIDS (4,4'-Diisothiocyano-2,2'-stilbenedisulfonic acid), significantly slow the pump (Kuang, Li et al. 2004; Riley, Winkler et al. 1996). There must also be a role for secondary Na^+ fluxes because amiloride, which blocks the Na^+/H^+ exchanger and at high concentrations the Na^+ channel ENaC (Epithelial Sodium Channel), also slows fluid transport (Liebovitch and Fischbarg 1982). Assembling these disparate observations into a clear model for endothelial pump function has been very challenging and is described below.

4. The Anion (Bicarbonate & Chloride) Secretion Model

Fluid secretion that is coupled to ion fluxes is dependent on active transport mechanisms to produce local osmotic gradients that move water across the cellular layer. This is best described for secretory glands and kidney reabsorption processes. More recently, evidence for direct coupling of water to ion fluxes in cotransporters has also been presented (Hamann, Kiilgaard et al. 2003; Meinild, Loo et al. 2000) and could have a significant role in fluid absorption across the intestinal mucosa (Loo, Zeuthen et al. 1996). The best tissue models for bicarbonate secretion are the gall bladder and pancreas. In both tissues transporters and anion channels have been identified in both basolateral and apical membranes that could provide the net transcellular bicarbonate flux that can unequivocally be measured. In contrast, several groups have measured HCO_3^- and Cl^- fluxes across the rabbit corneal endothelium. However, there is no consensus that a net anion flux can be generated (Bonanno 2003). Moreover, the basolateral membrane HCO_3^- permeability of the corneal endothelium is significantly higher than the apical permeability (Bonanno, Guan et al. 1999) and with the exception of apical anion channels; no apical HCO_3^- transporter has been identified.

Figure 1 shows a possible bicarbonate transport model for the corneal endothelium. The basolateral Na^+/K^+ ATPase creates a low intracellular $[\text{Na}^+]$ and high intracellular $[\text{K}^+]$, and in conjunction with K^+ channels a negative membrane potential of about -55 mV (Watsky and Rae 1991). On the basolateral side there are two bicarbonate transporters, a $1\text{Na}^+ : 2\text{HCO}_3^-$ cotransporter (*SLC4A4*, NBCe1; Sodium Bicarbonate Cotransporter electrogenic) and the $\text{Cl}^-/\text{HCO}_3^-$ exchanger (*SLC4A2*, AE2; Anion Exchanger), as well as a Na^+/H^+ exchanger (*SLC9A6*, NHE1; Sodium Hydrogen Exchanger). NBCe1 can directly load HCO_3^- into the cell. The Na^+/H^+ exchanger can indirectly load HCO_3^- into the cell because removal of protons favors formation of HCO_3^- from CO_2 , catalyzed by carbonic anhydrase II. Conversely, the $\text{Cl}^-/\text{HCO}_3^-$ exchanger is poised to remove HCO_3^- from the cell and add Cl^- . Moreover, a basolateral $\text{Na}^+:\text{K}^+:2\text{Cl}^-$ cotransporter helps load intracellular chloride to ~ 40 mM, above electrochemical equilibrium (~ 12 mM). On the apical side, at least two anion channels have been identified: CFTR (Cystic Fibrosis Transmembrane conductance Regulator) and CaCC (Calcium activated Chloride Channel). Anion permeability of both channels favors Cl^- vs HCO_3^- by $\sim 4:1$. There is no evidence for apical $\text{Cl}^-/\text{HCO}_3^-$ exchange. As such the model (see Figure 1) predicts that HCO_3^- is taken up on the basolateral side and efflux of HCO_3^- across the apical side is through anion channels driven by the negative membrane potential. Figure 1 shows an additional potential indirect route for apical HCO_3^- efflux. Because the influx mechanisms for HCO_3^- exceed direct efflux mechanisms and the differential must be converted to CO_2 (Bonanno and Giasson 1992), the CO_2 can diffuse in any direction and some will diffuse across the apical membrane where carbonic anhydrase IV could catalyze the conversion back to HCO_3^- and thereby contribute to a local buildup of osmotic pressure at the apical membrane. The following sections

discuss recent studies of several corneal endothelial transport mechanisms and their potential contribution to an anion secretion model.

4a. NKCC1

The role for Cl^- is unclear. Intracellular $[\text{Cl}^-]$ is about 40 mM in bovine endothelium (Srinivas, Bonanno et al. 1998), which is above electrochemical equilibrium. This is due to the presence of a bumetanide sensitive basolateral $\text{Na}^+:\text{K}^+:2\text{Cl}^-$ cotransporter (NKCC1) (Diecke, Zhu et al. 1998; Diecke, Wen et al. 2005; Jelamskii, Sun et al. 2000). Together with the apical Cl^- channels, the potential for Cl^- secretion exists. One study could not show any effect of the $\text{Na}^+:\text{K}^+:2\text{Cl}^-$ blocker bumetanide on rabbit corneal deturgescence (Riley, Winkler et al. 1997), however small, but significant effects were noted in a later study (Diecke, Wen et al. 2005). Certainly NKCC1 is involved in regulation of cell volume, however given the lack of clear evidence for a net transendothelial Cl^- flux, the role NKCC1 could have in the endothelial pump is uncertain. One suggestion is that Cl^- efflux through anion channels offsets the membrane hyperpolarizing effect of the electrogenic $1\text{Na}^+:2\text{HCO}_3^-$ cotransporter. By keeping the membrane potential relatively depolarized through Cl^- efflux, the driving force for continual $1\text{Na}^+:2\text{HCO}_3^-$ influx is maintained.

4 b. NBCe1

The dependence of endothelial fluid transport on active transport and the presence of bicarbonate suggest an important role for the $1\text{Na}^+:2\text{HCO}_3^-$ cotransporter (NBCe1). NBCe1 has two splice variants, pNBCe1 and kNBCe1, for pancreas and kidney type, respectively. The kidney proximal tubule form of NBC (kNBC) has a 1:3 stoichiometry, which favors cellular efflux of HCO_3^- , and the pancreas form of NBC (pNBC) has a 1:2 stoichiometry, which favors HCO_3^- influx. However, more recent studies have shown that the stoichiometry of either kNBC or pNBC can change depending on the cell type in which it is expressed (Gross, Hawkins et al. 2001). Previous reports indicated the pNBC variant was expressed in bovine (Sun, Bonanno et al. 2000), human (Sun and Bonanno 2003) and rat corneal endothelium (Bok, Schibler et al. 2001), and one report suggested both variants were expressed in human endothelium (Usui, Seki et al. 1999). Immunohistochemistry studies in cultured and fresh bovine, rat, and human endothelium indicate that NBCe1 exclusively locates to the basolateral membrane; however, one report (Diecke, Wen et al. 2004) suggests apical expression as well. NBCe1 specific siRNA knockdown reduced NBCe1 expression by 80-90% four days post transfection in cultured bovine corneal endothelial cells (Li, Sun et al. 2005). This treatment significantly reduced basolateral HCO_3^- permeability and also reduced the basolateral to apical HCO_3^- flux induced by basolateral to apical $[\text{HCO}_3^-]$ gradient. Importantly, apical HCO_3^- permeability and apical to basolateral HCO_3^- flux was unaffected by siRNA knockdown. This study shows that functional NBCe1 is only present on the basolateral membrane and that it is necessary for a large portion of basolateral HCO_3^- influx.

4c. Calcium Activated Chloride Channels

In bovine corneal endothelial cells, apical HCO_3^- permeability can be enhanced by increasing $[\text{Ca}^{2+}]_i$ via either activation of purinergic receptors (e.g., by ATP) or inhibition of the sarcoendoplasmic reticulum Ca^{2+} -ATPase (SERCA) (Xie, Zhang et al. 2002). In either case there is a release of Ca^{2+} from intracellular stores with concomitant Ca^{2+} entry from the extracellular bath through store-operated channels in a process called capacitive calcium entry (CCE). CCE agonists enhanced apical HCO_3^- permeability in Ca^{2+} -rich Ringer solution, but not in the absence of extracellular Ca^{2+} , which was blocked by the CCE inhibitor 2-aminoethoxydiphenyl borate (2-APB) (Zhang, Li et al. 2006; Zhang, Xie et al. 2002). ATP release from endothelial cells does occur and can be stimulated by injury or cell

volume increase (Srinivas, Mutharasan et al. 2001) indicating that this pathway could be important during cellular stress.

A likely candidate for the apical Ca^{2+} sensitive channel is CLCA (chloride channel, calcium activated)(Cunningham, Awayda et al. 1995). CLCA1 antiserum detected the 90kD band in corneal endothelium. Immunofluorescence staining showed an apical membrane location(Zhang, Li et al. 2006). In endothelial cells transfected with CLCA1 specific siRNA, CLCA1 expression was reduced by 80%. Activating CCE increased apical HCO_3^- permeability in siControl transfected cells, while having no effect in CLCA1 specific siRNA transfected cells. Baseline HCO_3^- permeability however, was not different between control and siRNA treated cells. These studies indicate that the calcium-activated chloride channel (CLCA1) is expressed in corneal endothelial cells and can contribute to Ca^{2+} dependent apical HCO_3^- permeability, but not resting permeability across the corneal endothelium. Since release of ATP activates the purinergic pathway, this channel may have a role during stress, e.g. inflammation, to enhance “Pump” activity.

4d. CFTR

Increasing intracellular [cAMP] by activating membrane adenylyl cyclases with forskolin stimulates an NPPB (5-nitro-2-(3-phenylpropylamin benzoic acid) and glibenclamide-inhibitable apical Cl^- and HCO_3^- permeability that has been identified as CFTR (Cystic Fibrosis Transmembrane conductance Regulator) in bovine corneal endothelium (Sun and Bonanno 2002). Knocking down CFTR expression using siRNA eliminated forskolin induced increases in apical Cl^- and HCO_3^- permeability (Li, Allen et al. 2008). However, the siRNA knockdown had no effect on baseline unstimulated anion permeability. Stimulation of CFTR can also induce a steady-state apical bath alkalinization. In CFTR siRNA treated cells, the baseline pH was similar to control, but stimulation did not produce an apical alkalinization. These findings indicate that cAMP induced increases in apical HCO_3^- permeability in bovine corneal endothelium are due to CFTR. However, CFTR does not have a major role in determining baseline apical chloride or HCO_3^- permeability. This is consistent with reports indicating that there are no corneal abnormalities in humans with cystic fibrosis and studies showing that a specific CFTR blocker had no significant effect on baseline corneal thickness (Diecke, Wen et al. 2004). CFTR may, like CLCA, have a role only during stimulated stressful conditions.

4e. CA IV

As described in figure 1, there is the potential for apical CO_2 flux contributing to net basolateral to apical HCO_3^- flux. This requires an apical carbonic anhydrase. An apical CAIV is present in bovine corneal endothelium (Sun, Li et al. 2008). The relatively membrane impermeant carbonic anhydrase inhibitor benzamide or CAIV siRNA knockdown reduced apical CO_2 fluxes due to an imposed $[\text{CO}_2]$ gradient by ~20%, however there was no effect on HCO_3^- permeability or HCO_3^- flux (Sun, Li et al. 2008). If a significant net flux of CO_2 occurred across the apical membrane with conversion to $\text{HCO}_3^- + \text{H}^+$ (figure 1), then blocking the apical CAIV would cause an alkalinization of the apical bath. However, apically applied benzamide and siRNA treated cells showed an acidification of the apical side relative to basolateral, which is inconsistent with a net cell to apical CO_2 flux. Because CAIV does not facilitate steady-state cell to apical CO_2 flux, apical HCO_3^- permeability, or basolateral to apical HCO_3^- flux, but apparently maintains apical side pH, CAIV may have a role in buffering the apical surface.

4f. Role of NBCe1: In Vivo Studies

A mouse knockout for NBCe1 exists, however NBCe1 knockouts have significant developmental and systemic abnormalities and die shortly after birth, limiting their

usefulness in analyzing the role of NBCe1 in the cornea. Therefore, to follow up on the previous *in vitro* studies, lentiviral vectors encoding NBCe1 specific shRNA were constructed and injected into the anterior chamber of live rabbits to determine if the bicarbonate fluxes produced by NBCe1 were needed to support corneal endothelial fluid transport *in vivo* (Liu, Cheng et al. 2010). This study showed that significant (up to 90%) endothelial NBCe1 knockdown could occur *in vivo*, however there was large variability in knockdown efficiency. If transendothelial HCO_3^- transport were an integral part of the endothelial pump, then knocking down NBCe1 should induce significant corneal swelling. Surprisingly, there was no effect on corneal thickness. However, relative to control eyes that were injected with a Lentivirus encoding a scrambled sequence shRNA, the eyes receiving the NBCe1 specific shRNA were more sensitive to the topical carbonic anhydrase inhibitor brinzolamide, i.e. there was more corneal swelling. These results were interpreted to suggest that the corneal endothelium has a large functional reserve (e.g., NBCe1 & CA activity), however they could also be interpreted to suggest that transendothelial HCO_3^- flux is not an integral part of the “Pump”. In summary, the recent studies presented above do not directly support the anion secretion model.

5. The Electro-osmosis Model

The conventional view of epithelial cell secretion and absorption of water is that epithelial cell layers create local osmotic differences in the lateral spaces between the cells and/or on the apical surfaces of cells within an unstirred layer. The osmotic gradients generated serve as the driving forces for water movement across the epithelium. However, in many epithelial cells, including the corneal endothelium, there is no evidence that these gradients exist. This has led to the consideration of other mechanisms, such as Electro-Osmosis (Sanchez, Li et al. 2002). In this process, cells generate a transepithelial potential (0.5 mV apical side negative in corneal endothelium), through the concerted action of apical anion channels (HCO_3^- and Cl^-), that draws counter-ions, i.e. Na^+ , through a paracellular pathway (the lateral space between cells) lined with fixed negative charges that make the paracellular pathway Na^+ specific. This produces electro-osmotic fluid coupling across the tight junction. Evidence for electro-osmosis and against other modes of fluid transport are recently reviewed (Fischbarg 2010). For electro-osmosis, a “leaky” junction with low specific resistance (20 ohm cm^2) must have a relatively intense current density. This high Na^+ current generates fluid movement by electro-osmotic coupling. The Na^+ current loop is complete due to the presence of apical Na^+ channels (ENaC) (Mirshahi, Nicolas et al. 1999). Of interest, apical membrane density of ENaC can be enhanced by SGK1 activity (serum glucocorticoid kinase), which has also been shown to increase Na/K-ATPase activity in corneal endothelium (Hatou, Yamada et al. 2009; Rauz, Walker et al. 2003). This could be interpreted in favor of electro-osmosis or the increased ENaC density may simply be needed to offset the hyperpolarizing effect of increased Na/K-ATPase activity in order to maintain a stable membrane potential. Moreover, glucocorticoids thin rabbit (Hara 1970), but not human corneas (Baum and Levene 1968). A major problem with electro-osmosis in corneal endothelium is that a significant reduction in endothelial cell density, which is a normal process of ageing, does not result in reductions in endothelial function or production of corneal edema. The integrated effect of the high current density would be dissipated since the total paracellular area is significantly decreasing with age.

6. Aquaporins

The role of aquaporins in fluid transport has been controversial. Knockout models of AQP1, the most ubiquitous aquaporin, showed essentially no phenotype unless the animal was stressed by withholding water (Agre 1998). Subsequent studies indicated that knockout of aquaporins had effects on secretory tissues if the rate of secretion was relatively high (Ma,

Fukuda et al. 2000; Ma, Song et al. 1999). Corneal endothelium has a high density of AQP1 located on both apical and basolateral membranes (Kuang, Yiming et al. 2004). In the knockout mouse, corneal thickness is thinner than wild type rather than the expected thicker more hydrated cornea if AQP1 was an important part of the “Pump”. The endothelial osmotic permeability is significantly reduced, as expected, and the rate of corneal thickness change in response to an osmotic challenge is slowed. These results suggest that AQP1 is present as a passive water pathway that speeds stromal hydration changes due to osmotic pressure fluctuations. In normal physiological situations this would include eye closure, which swells the cornea by ~4%, or exposure to hypo-osmotic conditions, e.g. swimming. This is consistent with the findings in other tissues that aquaporins do not have a role in secretion if the rate is relatively low.

7. Fluid Transport Agonists

The most highly studied fluid transport agonists in corneal endothelium are those that result in increased [cAMP]. Adenosine was the first agonist discovered (Dikstein and Maurice 1972) and was subsequently shown that it increased [cAMP] in endothelial cells (Riley, Winkler et al. 1996) through activation of A_{2b} receptors (Tan-Allen, Sun et al. 2005). Other agonists that increase [cAMP], e.g. forskolin or the phosphodiester inhibitor rolipram, also cause corneal thinning (Wigham, Turner et al. 2000). It is important to note that this could occur in de-epithelialized preparations so it was not due to the well-characterized corneal epithelial cAMP dependent Cl⁻ secretion (Klyce 1975). Besides being protective for corneal endothelial cells (Li, Allen et al. 2011), increased cAMP will phosphorylate and activate the apical CFTR channel leading to increased Cl⁻ and HCO₃⁻ flux (Sun, Zhai et al. 2003). It also relaxes endothelial cells leading to better cell-cell apposition and increased transendothelial resistance (Ramachandran and Srinivas 2010). ATP is also a potential fluid transport agonist. Purinergic receptor activation increased apical anion channel permeability as described above. Moreover, ATP released by the endothelial cells, can be broken down to adenosine by the presence of ectoATPases (Soltau, Zhou et al. 1993). Again, the presence of this physiologic response is probably in place to stimulate endothelial function during inflammatory, oxidative, or traumatic stress to the cell layer, but this will need to be tested.

8. Facilitated Lactate Transport

Often overlooked is the fact that in the *in vivo* cornea there are substantial bulk gradients of ions, e.g. glucose, HCO₃⁻ and lactate⁻ (Chhabra, Prausnitz et al. 2009). [HCO₃⁻] is higher in the anterior chamber than in the cornea, especially when the eyelid is open. Thus HCO₃⁻ will diffuse into the cornea passively in the opposite direction of the putative bicarbonate secretory mechanism. The cornea gets all of its glucose from the anterior chamber. Glucose in the cornea is consumed thereby maintaining an inward gradient for glucose diffusion. The high concentration of mitochondria in the endothelial cells would minimize glucose consumption allowing for supply to the very glycolytic keratocytes and epithelial cells. Overall, Riley (Riley 1969) has shown that 85% of glucose consumed in the cornea is converted to lactic acid. Therefore, for every 100 glucose molecules entering the cornea, 170 lactate molecules are produced. Klyce (Klyce 1981) demonstrated that the epithelium is impermeable to lactate and that the [lactate] in the cornea was about twice that in the aqueous humor, so there is a large gradient for lactate flux from cornea (~13 mM) to anterior chamber (~7 mM) across the endothelium. Lactate:H⁺ cotransport was demonstrated in the endothelium (Giasson and Bonanno 1994) and the monocarboxylate transporters (MCTs) responsible are being studied in our laboratory.

Corneal hypoxia increases [lactate] and causes corneal swelling (Klyce 1981) indicating that lactate is an important osmolyte. It follows that any process that facilitates lactate efflux

from the cornea will help maintain dehydration. Conversely, any interference with lactate efflux will cause corneal swelling. Figure 2 illustrates a model for lactate efflux across the endothelium that is facilitated by HCO_3^- buffering, carbonic anhydrase activity, $1\text{Na}^+ : 2\text{HCO}_3^-$ cotransport, and Na^+/H^+ exchange. An important role for buffering acid is supported by studies indicating that deturgescence can be maintained in the absence of HCO_3^- if a high concentration of other buffers are present (Doughty and Maurice 1988). Studies in our laboratory are currently examining the extent to which this process may contribute to the endothelial pump.

9. Conclusion

The corneal endothelial pump is an active transport dependent process that requires both Cl^- and HCO_3^- , but is highly dependent on HCO_3^- and facilitated by carbonic anhydrase activity. While a HCO_3^- secretory model for the pump has been preferred, there are several issues that argue against this interpretation. These include: the lack of apical anion transporters, equivocal measures of net anion fluxes from stroma to anterior chamber (probably due to the extremely leaky nature of the endothelium), no data indicating the presence of local osmotic gradients, a bulk fluid HCO_3^- gradient that is against the putative secretory mechanism, and the greater potential for a Cl^- secretory mechanism. Alternatives such as electro-osmosis are being considered, but this suffers from the fact that significant reductions in electro-osmotic potential, i.e. the paracellular space, do not have direct effects on fluid pumping. The lactate efflux mechanism is attractive, but requires significant experimental testing. Increasing endothelial cAMP enhances pump function by a combination of anion channel activation and greater barrier function. Further studies are needed to determine if this mechanism could be used as a therapy for corneal edema.

References

- Agre P. Aquaporin null phenotypes: the importance of classical physiology. *Proc. Natl. Acad. Sci. USA*. August. 1998 95:9061–9063. 1998. [PubMed: 9689031]
- Baum JL, Levene RZ. Corneal thickness after topical corticosteroid therapy. *Arch Ophthalmol*. 1968; 79(4):366–9. [PubMed: 5640319]
- Bok D, Schibler MJ, Pushkin A, Sassani P, Abuladze N, Naser Z, Kurtz I. Immunolocalization of electrogenic sodium-bicarbonate cotransporters pNBC1 and kNBC1 in the rat eye. *Am J Physiol Renal Physiol*. 2001; 281(5):F920–935. [PubMed: 11592950]
- Bonanno J, Guan Y, Jelamskii S, Kang X. Apical and basolateral $\text{CO}_2\text{-HCO}_3^-$ permeability in cultured bovine corneal endothelial cells. *Am. J. Physiol*. 1999; 277:C545–C553. [PubMed: 10484341]
- Bonanno JA. Identity and regulation of ion transport mechanisms in the corneal endothelium. *Prog Retin Eye Res*. 2003; 22(1):69–94. [PubMed: 12597924]
- Bonanno JA, Giasson C. Intracellular pH regulation in fresh and cultured bovine corneal endothelium. II. $\text{Na}:\text{HCO}_3$ cotransport and Cl/HCO_3 exchange. *Invest Ophthalmol Vis Sci*. 1992; 33:3068–3079. [PubMed: 1399410]
- Chhabra M, Prausnitz JM, Radke CJ. Modeling corneal metabolism and oxygen transport during contact lens wear. *Optom Vis Sci*. 2009; 86(5):454–66. [PubMed: 19357551]
- Cunningham SA, Awaysda MS, Bubien JK, Ismailov II, Arrate MP, Berdiev BK, Benos DJ, Fuller CM. Cloning of an Epithelial Chloride Channel from Bovine Trachea. *J. Biol. Chem*. 1995; 270(52): 31016–31026. [PubMed: 8537359]
- Diecke F, Zhu Z, Kang F, Kuang K, Fischbarg J. Sodium, potassium, two chloride cotransport in corneal endothelium: characterization and possible role in volume regulation and fluid transport. *Invest Ophthalmol Vis Sci*. 1998; 39:104–10. [PubMed: 9430551]
- Diecke FP, Wen Q, Iserovich P, Li J, Kuang K, Fischbarg J. Regulation of Na-K-2Cl cotransport in cultured bovine corneal endothelial cells. *Exp Eye Res*. 2005; 80(6):777–85. [PubMed: 15939033]

- Diecke FP, Wen Q, Sanchez JM, Kuang K, Fischbarg J. Immunocytochemical localization of Na⁺-HCO₃⁻ cotransporters and carbonic anhydrase dependence of fluid transport in corneal endothelial cells. *Am J Physiol Cell Physiol*. 2004; 286(6):C1434–42. [PubMed: 14960417]
- Dikstein S, Maurice DM. The metabolic basis to the fluid pump in the cornea. *J Physiol*. 1972; 221(1): 29–41. [PubMed: 4259586]
- Doughty MJ, Maurice D. Bicarbonate sensitivity of rabbit corneal endothelium fluid pump in vitro. *Invest Ophthalmol Vis Sci*. 1988; 29(2):216–23. [PubMed: 3123416]
- Fischbarg J. Fluid transport across leaky epithelia: central role of the tight junction and supporting role of aquaporins. *Physiol Rev*. 2010; 90(4):1271–90. [PubMed: 20959616]
- Fischbarg J, Lim J. Role of cations, anions, and carbonic anhydrase in fluid transport across rabbit corneal endothelium. *J. Physiol*. 1974; 241:647–675. [PubMed: 4215880]
- Giasson C, Bonanno JA. Facilitated transport of lactate by rabbit corneal endothelium. *Exp Eye Res*. 1994; 59:73–81. [PubMed: 7835399]
- Gross E, Hawkins K, Abuladze N, Pushkin A, Cotton CU, Hopfer U, Kurtz I. The stoichiometry of the electrogenic sodium bicarbonate cotransporter NBC1 is cell-type dependent. *J Physiol*. 2001; 531(Pt 3):597–603. [PubMed: 11251043]
- Hamann S, Kiilgaard JF, la Cour M, Prause JU, Zeuthen T. Cotransport of H⁺, lactate, and H₂O in porcine retinal pigment epithelial cells. *Exp Eye Res*. 2003; 76(4):493–504. [PubMed: 12634113]
- Hara T. Effect of topical application of hydrocortisone on the corneal thickness. *Exp Eye Res*. 1970; 10(2):302–12. [PubMed: 5484772]
- Hatou S, Yamada M, Mochizuki H, Shiraishi A, Joko T, Nishida T. The effects of dexamethasone on the Na, K-ATPase activity and pump function of corneal endothelial cells. *Curr Eye Res*. 2009; 34(5):347–54. [PubMed: 19401877]
- Hodson S, Miller F. The bicarbonate ion pump in the endothelium which regulates the hydration of rabbit cornea. *J. Physiol*. 1976; 263:563–577. [PubMed: 828203]
- Jelamskii S, Sun XC, Herse P, Bonanno JA. Basolateral Na⁺-K⁺-2Cl⁻ cotransport in cultured and fresh bovine corneal endothelium. *Invest. Ophthalmol. Vis. Sci*. 2000; 41:488–495. [PubMed: 10670480]
- Klyce S. Transport of Cl, Na, and water by the rabbit corneal epithelium at resting potential. *American Journal of Physiology*. 1975; 228:1446–1452. [PubMed: 165732]
- Klyce S. Stromal lactate accumulation can account for corneal oedema osmotically following epithelial hypoxia in the rabbit. *J Physiol (Lond)*. 1981; 321:49–64. [PubMed: 7338822]
- Kuang K, Li Y, Yiming M, Sanchez JM, Iserovich P, Cragoe EJ, Diecke FP, Fischbarg J. Intracellular [Na⁺], Na⁺ pathways, and fluid transport in cultured bovine corneal endothelial cells. *Exp Eye Res*. 2004; 79(1):93–103. [PubMed: 15183104]
- Kuang K, Yiming M, Wen Q, Li Y, Ma L, Iserovich P, Verkman AS, Fischbarg J. Fluid transport across cultured layers of corneal endothelium from aquaporin-1 null mice. *Exp Eye Res*. 2004; 78(4):791–8. [PubMed: 15037113]
- Kumagai AK, Glasgow BJ, Pardridge WM. GLUT1 glucose transporter expression in the diabetic and nondiabetic human eye. *Invest Ophthalmol Vis Sci*. 1994; 35(6):2887–94. [PubMed: 8188484]
- Kwok LS, Klyce SD. Theoretical basis for an anomalous temperature coefficient in swelling pressure of rabbit corneal stroma. *Biophys J*. 1990; 57(3):657–62. [PubMed: 2306510]
- Li J, Allen KT, Sun XC, Cui M, Bonanno JA. Dependence of cAMP mediated increases in Cl⁻ and HCO₃⁻ permeability on CFTR in bovine corneal endothelial cells. *Exp Eye Res*. 2008; 86(4): 684–90. [PubMed: 18325495]
- Li J, Sun XC, Bonanno JA. Role of NBC1 in apical and basolateral HCO₃⁻ permeabilities and transendothelial HCO₃⁻ fluxes in bovine corneal endothelium. *Am J Physiol Cell Physiol*. 2005; 288(3):C739–46. [PubMed: 15548570]
- Li S, Allen KT, Bonanno JA. Soluble adenylyl cyclase mediates bicarbonate-dependent corneal endothelial cell protection. *Am J Physiol Cell Physiol*. 2011; 300(2):C368–74. [PubMed: 21123735]
- Liebovitch L, Fischbarg J. Effects of inhibitors of passive Na⁺ and HCO₃⁻ fluxes on electrical potential and fluid transport across rabbit corneal endothelium. *Current Eye Research*. 1982; 2:183–186. [PubMed: 7151464]

- Liu C, Cheng Q, Nguyen T, Bonanno JA. Knockdown of NBCe1 in vivo compromises the corneal endothelial pump. *Invest Ophthalmol Vis Sci*. 2010; 51(10):5190–7. [PubMed: 20445126]
- Loo DD, Zeuthen T, Chandy G, Wright EM. Cotransport of water by the Na⁺/glucose cotransporter. *Proc Natl Acad Sci U S A*. 1996; 93(23):13367–70. [PubMed: 8917597]
- Ma T, Fukuda N, Song Y, Matthay MA, Verkman AS. Lung fluid transport in aquaporin-5 knockout mice. *J. Clin. Invest*. 2000; 105:93–100. [PubMed: 10619865]
- Ma T, Song Y, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS. Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. *J. Biol. Chem*. 1999; 274:20071–4. [PubMed: 10400615]
- Maurice, D. The Cornea and Sclera. Academic Press; New York: 1981.
- Meinild A-K, Loo DDF, Pajor AM, Zeuthen T, Wright EM. Water transport by the renal Na⁺-dicarboxylate cotransporter. *Am J Physiol Renal Physiol*. 2000; 278:F777–F783. [PubMed: 10807589]
- Mirshahi M, Nicolas C, Mirshahi S, Golestaneh N, d'Hermies F, Agarwal MK. Immunochemical analysis of the sodium channel in rodent and human eye. *Exp Eye Res*. 1999; 69(1):21–32. [PubMed: 10375446]
- Ramachandran C, Srinivas SP. Formation and disassembly of adherens and tight junctions in the corneal endothelium: regulation by actomyosin contraction. *Invest Ophthalmol Vis Sci*. 2010; 51(4):2139–48. [PubMed: 20019371]
- Rauz S, Walker EA, Murray PI, Stewart PM. Expression and distribution of the serum and glucocorticoid regulated kinase and the epithelial sodium channel subunits in the human cornea. *Exp Eye Res*. 2003; 77(1):101–8. [PubMed: 12823993]
- Riley M. Glucose and oxygen utilization by the rabbit cornea. *Experimental Eye research*. 1969; 8:193–200. [PubMed: 5786869]
- Riley M, Winkler B, Czajkowski C, Peters M. The roles of bicarbonate and CO₂ in transendothelial fluid movement and control of corneal thickness. *Invest. Ophthalmol. Vis. Sci*. 1995; 36:103–12. [PubMed: 7822137]
- Riley M, Winkler B, Starnes C, Peters M. Adenosine promotes regulation of corneal hydration through cyclic adenosine monophosphate. *Invest. Ophthalmol. Vis. Sci*. 1996; 37:1–10. [PubMed: 8550312]
- Riley M, Winkler B, Starnes C, Peters M. Fluid and ion transport in corneal endothelium: insensitivity to modulators of Na-K-2Cl cotransport. *Am. J. Physiol*. 1997; 273:C1480–C1486. [PubMed: 9374632]
- Sanchez JM, Li Y, Rubashkin A, Iserovich P, Wen Q, Ruberti JW, Smith RW, Rittenband D, Kuang K, Diecke FP, Fischbarg J. Evidence for a central role for electro-osmosis in fluid transport by corneal endothelium. *J Membr Biol*. 2002; 187(1):37–50. [PubMed: 12029376]
- Soltau JB, Zhou LX, McLaughlin BJ. Isolation of plasma membrane domains from bovine corneal endothelial cells. *Exp Eye Res*. 1993; 56(1):115–20. [PubMed: 8381748]
- Srinivas SP, Bonanno JA, Hughes BA. Assessment of Swelling-Activated Cl⁻ Channels Using the Halide-Sensitive Fluorescent Indicator 6-Methoxy-N-(3-Sulfopropyl)quinolinium. *Biophys. J*. 1998; 75(1):115–123. [PubMed: 9649372]
- Srinivas SP, Mutharasan R, Sun XC, Bonanno JA. Stretch-activated ATP release by corneal endothelium. *Invest Ophthalmol Vis Sci*. 2001; 42(4):S665.
- Sun XC, Bonanno JA. Expression, localization, and functional evaluation of CFTR in bovine corneal endothelial cells. *Am J Physiol Cell Physiol*. 2002; 282(4):C673–683. [PubMed: 11880256]
- Sun XC, Bonanno JA. Identification and cloning of the Na/HCO₃⁻ cotransporter (NBC) in human corneal endothelium. *Exp Eye Res*. 2003; 77(3):287–95. [PubMed: 12907161]
- Sun XC, Bonanno JA, Jelamskii S, Xie Q. Expression and localization of NaHCO₃ cotransporter in bovine corneal endothelium. *Am J Physiol Cell Physiol*. 2000; 279:C1648–C1655. [PubMed: 11029313]
- Sun XC, Li J, Cui M, Bonanno JA. Role of carbonic anhydrase IV in corneal endothelial HCO₃⁻ transport. *Invest Ophthalmol Vis Sci*. 2008; 49(3):1048–55. [PubMed: 18326729]

- Sun XC, Zhai CB, Cui M, Chen Y, Levin LR, Buck J, Bonanno JA. HCO₃⁽⁻⁾-dependent soluble adenylyl cyclase activates cystic fibrosis transmembrane conductance regulator in corneal endothelium. *Am J Physiol Cell Physiol*. 2003; 284(5):C1114–22. [PubMed: 12519749]
- Tan-Allen KY, Sun XC, Bonanno JA. Characterization of adenosine receptors in bovine corneal endothelium. *Exp Eye Res*. 2005; 80(5):687–96. [PubMed: 15862176]
- Usui T, Seki G, Amano S, Oshika T, Miyata K, Kunimi M, Taniguchi S, Uwatoko S, Fujita T, Araie M. Functional and molecular evidence for Na⁽⁺⁾-HCO₃⁻ cotransporter in human corneal endothelial cells. *Pflugers Arch*. 1999; 438:458–62. [PubMed: 10519138]
- Watsky MA, Rae JL. Resting Voltage Measurements of the rabbit corneal endothelium using patch-current clamp techniques. *Investigative Ophthalmology and Visual Science*. 1991; 32:106–111. [PubMed: 1987091]
- Wigham CG, Turner HC, Swan J, Hodson SA. Modulation of corneal endothelial hydration control mechanisms by Rolipram. *Pflugers Arch*. 2000; 440:866–870. [PubMed: 11041552]
- Winkler B, Riley M, Peters M, Williams F. Chloride is required for fluid transport by the rabbit corneal endothelium. *Am J Physiol*. 1992; 262:C1167–C1174. [PubMed: 1590358]
- Xie Q, Zhang Y, Zhai C, Bonanno JA. Calcium influx factor from cytochrome P-450 metabolism and secretion-like coupling mechanisms for capacitative calcium entry in corneal endothelial cells. *J Biol Chem*. 2002; 277(19):16559–66. [PubMed: 11867616]
- Zhang Y, Li J, Xie Q, Bonanno JA. Molecular expression and functional involvement of the bovine calcium-activated chloride channel 1 (bCLCA1) in apical HCO₃⁻ permeability of bovine corneal endothelium. *Exp Eye Res*. 2006; 83(5):1215–24. [PubMed: 16899243]
- Zhang Y, Xie Q, Sun XC, Bonanno JA. Enhancement of HCO₃⁻ Permeability across the Apical Membrane of Bovine Corneal Endothelium by Multiple Signaling Pathways. *Invest Ophthalmol Vis Sci*. 2002; 43(4):1146–1153. [PubMed: 11923259]

Research Highlights

- Review of corneal endothelial pump function
- Evidence supporting an anion transport based pump is limited
- siRNA and shRNA approaches provide new insights on endothelial function
- Evidence for alternate endothelial pump models including electro-osmosis & lactate flux

Figure 1.

Bicarbonate Secretion Model for Endothelial Pump. Fluid coupled anion secretion requires transendothelial net flux of Cl^- and/or HCO_3^- . The movement of net negative charge creates a small potential difference (0.5 mV, apical side negative) that attracts Na^+ through the paracellular pathway & across the tight junction (TJ). The net flux of NaHCO_3^- and/or NaCl constitutes the osmotic driving force for water movement. HCO_3^- uptake on the basolateral membrane is through the actions of the $1\text{Na}^+:2\text{HCO}_3^-$ cotransporter (NBCe1) and the Na^+/H^+ exchanger (NHE1). Cl^- uptake is primarily via the $\text{Na}^+:\text{K}^+:2\text{Cl}^-$ cotransporter (NKCC1) and the $\text{Cl}^-/\text{HCO}_3^-$ exchanger (AE2). The high intracellular $[\text{Cl}^-]$ and $[\text{HCO}_3^-]$, together with the negative membrane potential can then drive anions across the apical membrane through anion selective channels. An additional route for net HCO_3^- flux is for the high intracellular $[\text{HCO}_3^-]$ to be converted to CO_2 , facilitated by carbonic anhydrase II (CAII), apical CO_2 diffusion and conversion back to HCO_3^- , facilitated by carbonic anhydrase IV (CAIV) at the apical surface. This pathway is less attractive because it does not contribute to the transendothelial potential.

Figure 2.

Facilitated Lactate Transport Model. The bulk movement of lactic acid from cornea to anterior chamber via transcellular Monocarboxylate Cotransporters (MCTs) could contribute to net fluid movement out of the cornea. Intracellular buffering by the high intracellular $[\text{HCO}_3^-]$, provided by the $1\text{Na}^+:2\text{HCO}_3^-$ (NBCe1), Na^+/H^+ exchanger (NHE1), and carbonic anhydrase II work to facilitate basolateral lactate: H^+ entry. Apical efflux through an MCT is facilitated by robust apical surface buffering, which is supplied by aqueous humor HCO_3^- and apical surface carbonic anhydrase IV.