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Molecular mechanisms underlying the corneal endothelial pump

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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.

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 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 H_2O/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 path length, 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 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

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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 et al., 1995). Because Cl^- removal did not cause initial swelling, the early pump model was described as an exclusiveHCO_3^- secretory mechanism. Subsequent studies indicated that Cl^- was important for maintaining the pump (Winkler et al., 1992), suggesting that an anion exchanger, (Cl^-/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 et al., 2004a; Riley 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 et al., 2003; Meinild et al., 2000) and could have a significant role in fluid absorption across the intestinal mucosa (Loo 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 et al., 1999) and with the exception of apical anion channels; no apical HCO_3^- transporter has been identified.

Fig. 1 shows a possible bicarbonate transport model for the corneal endothelium. The basolateral Na^+, K^+ ATPase creates a low intracellular [Na^+] and high intracellular [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 $1Na^+$:$2HCO_3^-$ cotransporter (*SLC4A4*,NBCe1; Sodium Bicarbonate Cotransporter electrogenic) and the Cl^-/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 Cl^-/HCO_3^- exchanger is poised to remove HCO_3^- from the cell and add Cl^-. Moreover, a basolateral Na^+:K^+:$2Cl^-$ 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 ~4:1.There is no evidence for apical Cl^-/HCO_3^- exchange. As such the model (see Fig. 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. Fig. 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

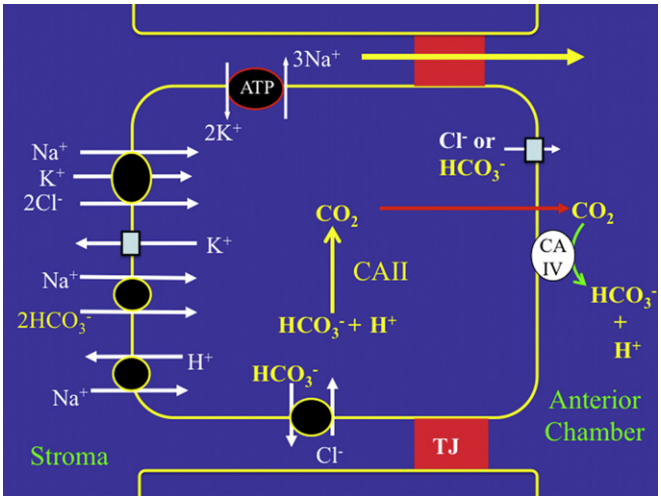

Fig. 1. Bicarbonate Secretion Model for Endothelial Pump. Fluid coupled anion secretion requires transendothelial net flux of Cl^- and/or HCO_3^-. The movement of net negative charge creates a small potential difference (0.5 mV, apical side negative) that attracts Na^+ through the paracellular pathway & 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 $1Na^+$:$2HCO_3^-$ cotransporter (NBCe1) and the Na^+/H^+ exchanger (NHE1). Cl^- uptake is primarily via the Na^+:K^+:$2Cl^-$cotransporter (NKCC1) and the Cl^-/HCO_3^- exchanger (AE2). The high intracellular [Cl^-] and [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 [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.