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FURTHER EVIDENCE FOR LOCAL OSMOTIC COUPLING IN THE RABBIT CORNEA

KENNETH R. MAYES

Unit of Electron Microscopy, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN (U.K.)

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Summary

1. The present experiments measure net fluxes of fluid, Cl^- and HCO_3^- across de-epithelialised rabbit corneas clamped between half chambers and bathed in Ringer solutions.

2. Net fluxes of HCO_3^- and fluid occurred together across the cornea from stroma to aqueous when HCO_3^- and CO_2 were present in the bathing solution.

3. No net trans-corneal Cl^- flux was found

4. The initiation of fluid flow in the presence of HCO_3^- and CO_2 cannot be accounted for by bulk-phase osmotic flow across the cornea.

Introduction

There is an active process in the rabbit corneal endothelium which is responsible for stromal dehydration [1,2] and which opposes the passive tendency for stromal swelling [3,4]. The existence of a process in the endothelium which is capable of creating actual bulk flow of fluid across the de-epithelialised cornea (in the direction stroma to aqueous) was demonstrated by Maurice [5].

Hodson and Miller [6] showed that there is a bicarbonate pump in rabbit corneal endothelium which translocates HCO_3^- across the endothelium from stroma to aqueous. This bicarbonate pump operates both in vitro [6] and in vivo [7] and has been shown to be responsible for regulating stromal hydration [6].

Specular microscope experiments based on those of Maurice [5] produced evidence that in corneas without an epithelium the trans-endothelial net fluid flux is coupled to the net HCO_3^- flux by a local osmotic mechanism [8].

The present paper describes experiments which measure fluid, HCO_3^- and Cl^- fluxes across de-epithelialised corneas mounted between two half cham-

bers. This investigation provides further evidence that there is local osmotic coupling between the trans-endothelial HCO_3^- and fluid fluxes observed across the de-epithelialised corneas.

Materials and Methods

De-epithelialised corneas were used in these experiments. They were taken from ex-breeding Dutch rabbits (age 2—2½ years) and were dissected atraumatically together with a scleral rim [2]. The scleral rim was then trimmed from the preparation without touching the endothelium. Each cornea was clamped between two perspex half chambers of circular cross-section, diameter 6 mm and volume 250 μl. A stainless-steel mesh (mesh size 1 mm^2) supported the stromal surface. Silicone grease was applied around the cut edge of the cornea to eliminate water loss by that route.

The system used is shown in Fig. 1. The tubing consisted of an inner polythene tube (for low water permeability) and an outer nylon tube (for low CO_2 permeability). Preliminary experiments showed that Ringer solution in both half chambers had a total CO_2 content some 3% below fresh Ringer solution values but that no further drop in total CO_2 occurred over the experimental period. Before filling the system it was flushed with a 5% CO_2/88% N_2/7% O_2 gas mixture. The inlet tubes to the chambers were of 1 mm bore, but the chamber outlets were large (4 mm bore) to prevent a high hydrostatic pressure developing in the chambers during filling

Filling the chambers

After clamping the cornea between the half chambers, both halves of the

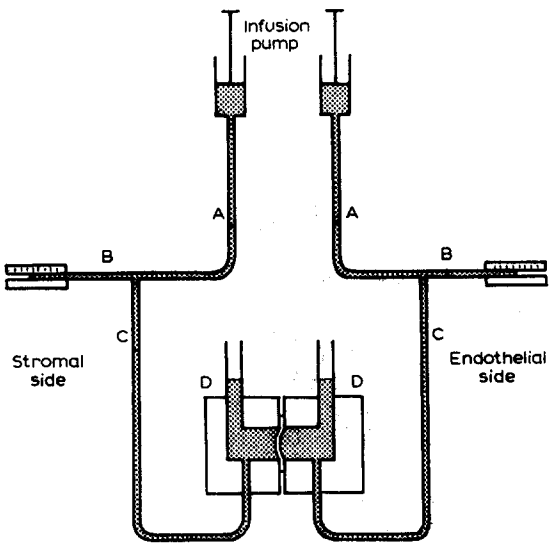

Fig. 1. A schematic diagram of the apparatus used to measure fluid and ion fluxes. A, B, C and D are clamping positions used during filling and adjustment of the system to minimise the hydrostatic pressure applied to the cornea.

system were filled simultaneously with Ringer solution from syringes mounted in a Vickers Treonic IP3 infusion pump at a rate of 15 ml · h^{-1}. The Ringer solution had the composition: NaCl (106 mM), $NaHCO_3$ (37 mM), KCl (6.7 mM), $MgSO_4$ (0.6 mM), NaH_2PO_4 (5.55 mM), $CaCl_2$ (0.56 mM), glucose (4.45 mM), reduced glutathione (1 mM), equilibrated with a gas mixture of 5% CO_2/88% N_2/7% O_2 [2]. This solution is referred to as 'full Ringer solution'. The tubes were clamped at B (Fig. 1) during filling. After filling the chambers and unclamping at B, first the outlets (D) and then the inlets (C) were clamped. Clamping was always performed first, and unclamping last, on the endothelial side of the system. The chamber was lowered into a 35° C water bath and the meniscus levels in the Hamilton syringe barrels were adjusted using the syringes of the infusion pump. The tubes were finally clamped at A and opened at C. The Hamilton syringe barrel outlets were adjusted in height so that the endothelial side was slightly (3—8 mm) higher than the stromal side. Histological examination of a flat mount of the endothelium showed that no obvious damage was done to the cells by this procedure.

Fluid flow measurement

Fluid flows across the preparation were measured directly by noting (to the nearest 0.05 μl) the movement of the meniscus in each 10 μl Hamilton syringe barrel.

Total CO_2 and Cl^- concentration measurement

Total CO_2 was used as a measure of HCO_3^- concentration because at pH 7.4, 96% of the total CO_2 is in the form of HCO_3^-. After fluid movement had been noted for 15—20 min the chamber contents were aspirated via the unclamped outlets. 10-μl samples of the chamber contents were analysed in a Beckman Cl/CO_2 analyser without loss of total CO_2 from the samples. A correction factor for a buffering effect (1.19 ± 0.01) was applied to the total CO_2 measurements [7,8].

Control experiments with denuded stroma

Denuded stroma was mounted between the half chambers to demonstrate that any fluid or ion fluxes found in the above experiments were due to the presence of an endothelium. 3—8 mm H_2O hydrostatic pressure was applied to the 'aqueous-side' of the denuded stroma. Any fluid fluxes were noted and chamber contents were analysed for Cl^- and total CO_2 concentration after 30 min.

Control experiments with bicarbonate- and carbon dioxide-free Ringer solution

In these experiments the solution bathing the cornea was changed every 30—45 min so that full Ringer solution was alternated with a HCO_3^-- and CO_2-free Ringer solution. The composition of HCO_3^-- and CO_2-free Ringer solution was: NaCl (140 mM), KCl (6.6 mM), $MgSO_4$ (0.6 mM), Na_2HPO_4 (5.55 mM), $CaCl_2$ (0.56 mM), glucose (4.45 mM), reduced glutathione (1 mM) [6]. Fluid fluxes were noted with de-epithelialised corneas mounted between the half chambers.

Results

No net fluid movement occurred in the control experiments with denuded stroma clamped between the half chambers and total CO_2 and Cl^- were both uniformly distributed on each side of the cornea (n = 3).

In contrast to this, a trans-corneal fluid flux (8.4 ± 1.8 $\mu l \cdot cm^{-2} \cdot h^{-1}$, range 5.7—10.7 $\mu l \cdot cm^{-2} \cdot h^{-1}$; n = 5) was demonstrated with de-epithelialised corneas. The direction of fluid flow was from stromal chamber to endothelial chamber (from stroma to aqueous) (Fig. 2). Significant differences in total CO_2 concentrations across the de-epithelialised corneas were found in all of these experiments (P = 0.02—0.01; 0.02; 0.01—0.001; 0.02; 0.02—0.01) with the total CO_2 concentrations 1.2—2.4 mM greater in the endothelial chamber than in the stromal chamber. This difference in total CO_2 concentration represents a net HCO_3^- flux of 1.20 ± 0.46 · 10^{-6} equiv · $cm^{-2} \cdot h^{-1}$ (n = 5) from the stromal chamber to the endothelial chamber (from stroma to aqueous). The Cl^- concentration was the same on both sides of the cornea. The concentration of HCO_3^- in the translocated fluid is 0.15 equiv · l^{-1}, compared to an initial concentration in the Ringer solution of 0.037 equiv · l^{-1}.

The initiation of fluid flux occurred 18 ± 3 min (range 11—30 min) after filling the chambers with full Ringer solution.

Fig. 3 illustrates the effect of bathing the preparation alternately in full Ringer solution (containing HCO_3^- and CO_2) and in HCO_3^-- and CO_2-free Ringer solution. Initial transient meniscus movements can occur (probably due to slight

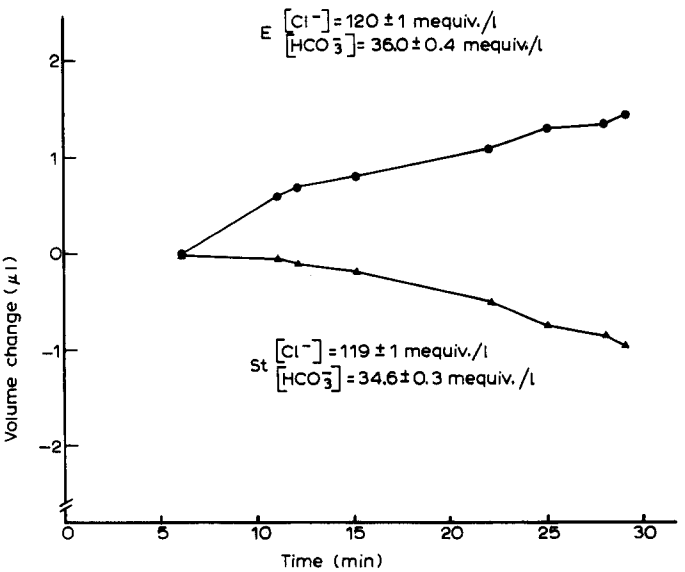

Fig. 2. De-epithelialised cornea clamped between the half chambers and bathed in full Ringer solution. Volume changes of fluid in the half chambers were measured by noting movements of the chamber outlet menisci in the 10-µl Hamilton syringe barrels. •, endothelial chamber meniscus level; ▲, stromal chamber meniscus level. The outlet menisci were initially adjusted to arbitrary levels in the syringe barrels = 0 µl in the figure. Symmetrical movement of the menisci indicates a net flux of fluid from stromal chamber to endothelial chamber. The figures shown are total CO_2 and Cl^- concentrations (± S.E.) of chamber contents after 30 min (E, endothelial chamber; St, stromal chamber).

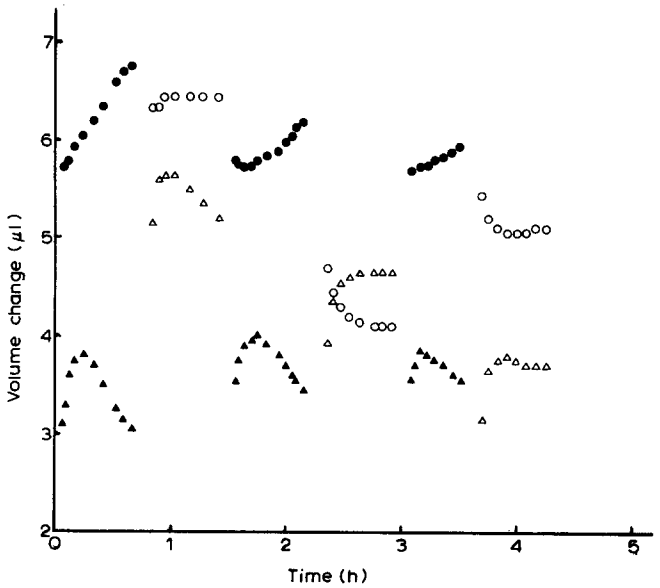

Fig. 3. De-epithelialised cornea clamped between the half chambers and bathed alternately in full Ringer solution and in bicarbonate- and carbon dioxide-free Ringer solution. Volume changes of fluid in the half chambers were measured by noting movements of chamber outlet menisci in the 10-µl Hamilton syringe barrels. Outlet menisci were adjusted to arbitrary levels in the syringe barrels after each solution change. ○ and ● endothelial chamber meniscus levels; △ and ▲, stromal chamber meniscus levels; closed symbols, full Ringer solution in chambers; open symbols, bicarbonate- and carbon dioxide-free Ringer solution in chambers.

pressure differences in the half chambers after filling) but in HCO_3^-- and CO_2-free Ringer solution movements generally cease. In full Ringer solution (that is, in the presence of HCO_3^- and CO_2) the transients are replaced by a net movement from stromal side to endothelial side.

Discussion

The net HCO_3^- flux found here ($1.20 \pm 0.46 \cdot 10^{-6}$ equiv $\cdot$ cm$^{-2} \cdot$ h^{-1}) agrees well with previous values [6—8] though it is smaller than that reported by Hull et al. [9]. No net Cl^- flux was found. The fluid flux, 8.4 ± 1.8 µl $\cdot$ cm$^{-2} \cdot$h^{-1} ($n = 5$) is of a similar magnitude to published values [5,8,10].

Not net fluxes occur across denuded stroma. Net fluxes of HCO_3^- and fluid occur together across the de-epithelialised cornea in the direction stromal side to endothelial side (Fig. 2). Furthermore, the fluid flux is observed only when HCO_3^- and CO_2 are present in the bathing medium (Fig. 3) and the bicarbonate pump is switched on [6]. The net fluid flux observed in the present experiments is related to endothelial bicarbonate pump activity. These findings lead to the question of the nature of coupling between the HCO_3^- and fluid fluxes.

The observed mean initiation time for fluid flow was 18 ± 3 min. HCO_3^- is actively transported across the cornea and could set up an osmotic gradient and create fluid flow. The following calculation shows that such bulk-phase osmosis cannot account for fluid flows of the observed magnitude (8.4 µl $\cdot$ cm^{-2} . h^{-1}) after a period of 18 min.

The concentration difference across the cornea necessary to give an osmotic

fluid flux of 8.4 $\mu l \cdot cm^{-2} \cdot h^{-1}$ can be calculated using the equation for volume flow across a membrane [11] which is applicable to the cornea [12,13]:

$$J_v = Lp\ \Delta P - \sigma LpRT\ \Delta C \quad (1)$$

where J_v is the fluid flux, σ is the reflection coefficient for the endothelium for $NaHCO_3$ (0.59) [13], Lp is the hydraulic conductivity calculated for endothelium plus full-thickness stroma ($1.18 \cdot 10^{-11}$ $cm^3 \cdot s^{-1} \cdot dyne^{-1}$) [12], R is the gas constant, T is the absolute temperature, ΔC and ΔP are the concentration and hydrostatic pressure differences, respectively, across the cornea. The hydraulic component of J_v is negligible (about $3 \cdot 10^{-2}$ $\mu l \cdot cm^{-2} \cdot h^{-1}$) so that ΔC can be calculated from:

$$\Delta C = J_v / \sigma LpRT \quad (2)$$

The calculated value of ΔC required to create an osmotic fluid flux of the observed magnitude is $1.3 \cdot 10^{-2}$ osmol $\cdot l^{-1}$ and would be produced after the movement of $1.6 \cdot 10^{-6}$ osmol of mass from the stromal chamber to the endothelial chamber. With the measured HCO_3^- net flux rate ($1.2 \cdot 10^{-6}$ equiv $\cdot$ $cm^{-2} \cdot h^{-1}$), assuming an accompanying cation and correcting for the area exposed in the experiments (giving an ion flux of $11.2 \cdot 10^{-9}$ osmol . min^{-1}), this mass would be translocated in 143 min. The value of Lp used in the calculation may be underestimated [14] and the time for osmotic flow could be as low as 53 min. The initiation of fluid flux was observed 18 ± 3 min after the beginning of the experiments and so it cannot be accounted for by bulk-phase osmosis. Initial transient meniscus movements do occur (Fig. 3) and these may mask the actual onset of net fluid flow. The measured value for net fluid flow initiation time (mean 18 min) may be larger than the true figure.

Osmotic flow between the bulk-phase solutions does not provide a mechanism for salt-water coupling in the present experiments. Coupling between the HCO_3^- and fluid fluxes probably occurs within the compartment formed by the cornea plus unstirred layers. Models exist for such local coupling in epithelia [15,16] and require the active generation of a local osmotic gradient within a central compartment. This compartment has been interpreted as being the cell interior [17] or the intercellular space [16,18]. Recently Klyce and Russel [14] have shown theoretically that gradients of solute concentration within the corneal stroma can provide for solute-solvent coupling in the cornea. The present results, together with those of Mayes and Hodson [8], provide evidence for local osmotic coupling in the rabbit cornea.

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