Biochimica et Biophysica Acta, 646 (1981) 71–76
Elsevier/North-Holland Biomedical Press

BBA 79340

OSMOTIC WATER PERMEABILITY OF RABBIT CORNEAL ENDOTHELIUM AND ITS DEPENDENCE ON AMBIENT CONCENTRATION

LARRY S. LIEBOVITCH, JORGE FISCHBARG and RONALD KOATZ

Departments of Physiology and Ophthalmology, College of Physicians and Surgeons, Columbia University, New York, NY 10032 (U.S.A.)

(Received December 8th, 1980)
(Revised manuscript received April 9th, 1981)

Key words: Permeability; Osmotic gradient; (Rabbit corneal endothelium)

We measured the fluid flow osmotically induced by sucrose concentration differences across the isolated rabbit corneal endothelium to determine if its osmotic water permeability, P_{os}, depends on the concentrations on both sides as well as on the concentration difference across that tissue. We found that when the osmotically induced fluid flow went from stroma to aqueous, P_{os} decreased from 35 ± 4 μm/s to 20 ± 3 μm/s when an additional 20 mosM sucrose was added to the solutions on both sides of the endothelium. However, when the osmotically induced fluid flow was towards the stroma, P_{os} remained practically unchanged, (28 ± 4 μm/s vs. 31 ± 5 μm/s), when additional 20 mosM sucrose was present on both sides. These changes in the measured permeability are consistent with the possibility that sucrose would be swept into the intercellular channels by the osmotically induced fluid flow. We also confirmed that an osmotic gradient can 'prime' the fluid pump. After a gradient was removed, gradients which had previously induced flow from stroma to aqueous caused an increase in the basal fluid pump rate, while gradients in the opposite direction decreased that fluid pump rate.

Introduction

The corneal stroma is bordered by the stratified epithelial cell layer on the tear side and by the endothelial cell layer on the aqueous side. The stroma can absorb fluid, swell, and lose its transparency. The corneal endothelium has been shown [1,2] to be able to actively transport fluid. Thus, the endothelium is able to pump fluid out of the stroma into the aqueous, thereby maintaining the stroma at the relatively low level of hydration required for corneal transparency.

The values experimentally measured for the osmotic permeabilities of the tissue layer and of the cell membranes are important in order to determine the mechanism for solute-solvent coupling in this and other leaky epithelia (see Ref. 3). One of the elements which may make such measurements difficult is the presence of unstirred layers surrounding the epithelia, as exemplified in a recent numerical evaluation of these effects [4]. Moreover, aside from the unstirred layers outside of the epithelium proper, the convection of solutes into intercellular spaces within the epithelium driven by the osmotic flow can also affect those measurements [4,5]. Therefore, the experiments presently reported on were done in order to gauge the extent and magnitude of such sweeping-in effects.

Materials and Methods

Dissection and mounting. Male albino rabbits weighing 2.5–3 kg were killed by an overdose of sodium pentobarbital injected into the marginal vein of the ear. The eyes were excised and the corneal epithelia scraped off with a razor blade until the bare stroma presented typical 'ground glass' appearance. Using dissection techniques previously described [6] the globe, held by its conjunctiva, was

cut and dissected away so that only the endothelium and its supporting stroma and sclera were left.

Experimental solutions. Unless otherwise stated the 300 mosM medium 1 [7] contained 110.4 mM NaCl, 39.2 mM $NaHCO_3$, 3.8 mM $KHCO_3$, 1.0 mM KH_2PO_4, 0.8 mM $MgSO_4 \cdot 7\,H_2O$, 1.7 mM $CaCl_2 \cdot 2\,H_2O$, 6.9 mM glucose, and 5.1 mM sucrose. Additional amounts of sucrose were added as required to this medium 1 to form solutions of the desired concentrations. The osmolarity of these solutions was measured on occasion and found to have the desired value to within the ±1 mosmol/kg accuracy of an Osmette-A (Precision Systems, Inc.).

Measurement of fluid movements. The cornea was mounted in a chamber surrounded by a 36.8°C constant temperature bath and connected to a previously described apparatus that automatically measures and records fluid movements across the tissue [7]. One cornea from each rabbit was mounted in a fluid measurement chamber and bathed by medium 1 on both sides. The other cornea was mounted in another chamber but bathed on both sides by medium 1 to which an additional 20 mM sucrose had been added. Approximately one hour later, the stromata of both corneas were fully swollen with water and the fluid flow (J_v) from the stromal towards the aqueous side represented the basal rate of fluid transport by the endothelial fluid pump. After that, the solution bathing a given side of the cornea was changed at suitable intervals so that an osmotic gradient (Δc) due to a sucrose concentration difference of 0, 5, 10, 15, or 20 mM/l was imposed across the cornea. In the first series of experiments the aqueous bathing solutions was made hypertonic while in the second series the stromal bathing solution was made hypertonic by those same amounts. However, because of the architecture of the chamber, changing the stromal bathing solution involved a higher risk of damaging the delicate endothelial cell layer. As a consequence, fewer corneas survived through the second series of experiments. After each solution substitution, the maximum change in fluid flow, ΔJ_v, was determined. The osmotic permeability P_{os} was then determined; by definition, $P_{os} = \Delta J_v/(\bar{V}_w \Delta c)$ where $\bar{V}_w = 18\ cm^3/M$ is the molar volume of water. For the units of measurement used here: P_{os} (in units of μm/s) = 154 · ΔJ_v (in units of μl/h per cm^2)/Δc (in units of mosM). For those familiar with c.g.s. units, we note that $0.07 \times P_{os}$ (in units of μm/s) gives the value of the hydraulic conductivity, Lp, in units of 10^{-12} cm^3/s per dyn.

Results

In the first series of experiments the aqueous solution was made hypertonic so that the osmotic flow thus induced across the endothelium was in the same direction as that of the fluid driven by the physiological pump (Fig. 1). We found that when the endothelium was initially bathed by medium 1 on both sides, then P_{os} was 35 ± 4 (S.E.) μm/s. However, when the solutions initially bathing both sides of the endothelium contained an additional 20 mosM sucrose, P_{os} was 20 ± 3 μm/s. Thus the additional sucrose present on both sides of the endothelium lowered (Student's t-test, $P < 0.004$) the measured endothelial water permeability.

In the second series of experiments, the cornea was initially bathed as before, but now the stromal side was subsequently made hypertonic. Thus the osmotically induced fluid flow used to determine P_{os} was now oriented in a direction opposite to that in the first series of experiments. In this second series, we found that P_{os} = 28 ± 4 μm/s as determined from corneas initially bathed on both sides with medium 1 did not differ significantly (Student's t-test, $P < 0.7$) from the P_{os} = 31 ± 5 μm/s found for corneas initially bathed on both sides with the additional sucrose.

In both series of experiments it took 10 to 30 min after the solutions were changed for the new rate of fluid movement to reach its steady state. To help test whether this latency originated indeed in the tissue or was rather an artifact of our fluid flow measurement system, we performed osmotically-induced flow experiments where the cornea was replaced by an artificial membrane (Fisher 8-666-19). This cellulose dialysis membrane has a nominal 6 000 mol.wt. cut-off, so that sucrose (M_r 342) concentration differences of 100 to 1 000 mosM were required to drive fluid flows large enough to be easily measured. We found that the attainment of the new steady state of fluid flow across the artificial membrane occurred within 2 min after the imposition of the osmotic gradient. This supports the idea that the much longer latencies observed in the corneal experiments were not artifacts of the measurement procedure.

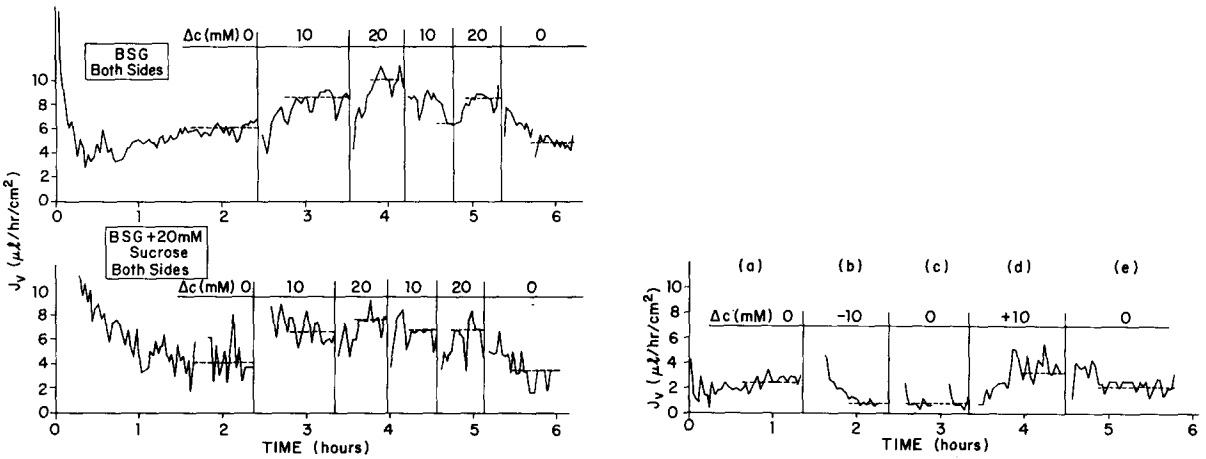

Fig. 1. Corneas from the same rabbit were denuded of epithelium. The remaining endothelium and supporting stroma were initially bathed in either medium 1 (BSG: basal salts plus glucose, top) or medium 1 with an addition of 20 mosM sucrose on both sides (bottom). The net fluid flow towards the aqueous J_v is shown as a function of time when solutions of additional sucrose concentration Δc were substituted on the aqueous side of the endothelium.

Fig. 2. Evidence that osmotic gradients can prime the endothelial fluid pump. After the cornea stabilized (a) the stroma was made hypertonic with respect to the aqueous, which reduced the net fluid flow towards the aqueous (b). However, when that sucrose osmotic gradient was removed by making both bathing solutions identical, the fluid flow did not return to its pre-gradient level (c). Similarly, when the aqueous solution was made hypertonic (d) the net fluid flow increased, but did not return to its pre-gradient level (c) after the gradient was removed (e).

It was also found that the 'history' of the osmotic gradients across a given preparation exerts an interesting influence on the results. The water permeabilities cited above were all measured after solution substitution had been performed that increased the absolute value of the concentration difference across the endothelium. On the other hand, when the absolute value of that concentration gradient was lowered, the reduction in fluid flow was less than the previous enhancement produced by the same osmotic gradient in 16 out of 21 trials (binomial test, $P < 0.014$). The fluid flows enhanced by the initial osmotic gradient (regardless of direction of flow) averaged 2.1 ± 0.1 (S.E.) μl/h per cm^2 per 10 mosM concentration difference while their subsequent reductions averaged 1.3 ± 0.3 μl/h per cm^2. This effect is illustrated by the experiment shown in Fig. 2.

Thus, osmotic gradients can 'prime' the endothelial fluid pump; namely, after an osmotic gradient is removed, water flow can remain enhanced in the direction originally stimulated by the osmotic gradient. Priming of the pump after the aqueous bathing solution had been temporarily made hypertonic (by the addition of 3.3 mosM 40 000-dalton dextran) has been previously reported [8]. In contrast, and just as one would intuitively expect, no such priming effects were observed here when osmotic gradients were used to drive water flow across the cellulose dialysis membrane.

Discussion

A. Asymmetry of effects

We found that, when the osmotically induced fluid flow went from stroma to aqueous, the osmotic permeability of the endothelium declined when the sucrose concentration in both bathing solutions was increased. On the other hand, when the osmotically induced fluid flow was in the opposite direction (that is, towards the stroma), there was no significant change of water permeability with increasing sucrose concentrations in both bathing solutions. These changes in the measured permeability can be interpreted as being due to the effects produced by the sweeping of sucrose into (or out of) the intercellular channels by the osmotically induced fluid flow. Con-

sider the first series of experiments where the direction of the osmotically induced fluid flow was towards the aqueous. The fluid flow would sweep solute (salts and sucrose) from the stromal bathing solution into the intercellular channel through its open stromal end. Water can continue to flow out of the channel either through the cell membrane or through the junction at the aqueous end of the tissue. However, the junction can impede the passage of solutes. Since the molecule of sucrose are larger than the small ions in the solution we expect the effects due to the restrictive tight junction to be more significant for sucrose than for the small ions. The fluid flow will sweep the sucrose into the channel which will accumulate at the intercellular channel terminus of the 'leaky' tight junction. A steady state will be reached when the flow of sucrose convected into the channel by the water flow is equal to the diffusional flow of sucrose back out of the channel down its concentration gradient that has now formed within the channel. As shown in Fig. 3, this buildup of sucrose within the intercellular channel will have two effects on fluid flow across the endothelium. First, the now elevated concentration at the intercellular channel terminus of the tight junction reduces the concentration difference between that location and the hypertonic aqueous. Thus the fluid flow driven through the tight junction by that concentration difference is reduced. Second, the total intracellular concentration can be assumed to be equal to the average of the environmental concentrations bordering the cell weighted by the cell membrane surface area and water permeability facing each concentration (for example, see Ref. 9). The buildup of sucrose within the intercellular channel would then be expected to raise the average environmental and therefore the total intracellular concentration. Thus the intracellular concentration would now be closer to that of the hypertonic aqueous so that the concentration difference driving the fluid flow across the apical (or aqueous-facing) cell membrane would also be reduced. At higher stromal sucrose concentrations, more sucrose will in turn be swept into and trapped within the extracellular channel. Therefore, with increased stromal sucrose concentrations, both the paracellular fluid flow through the junction and the transcellular fluid flow across the apical membrane should decrease so that the water permeability measured across the endothelium should decrease. In agreement with such expectation, we found here that the water permeability decreased by 43% from P_{os} = 35 ± 4 μm/s to P_{os} = 20 ± 3 μm/s when the stromal sucrose concentration was raised from 0 to 20 mM.

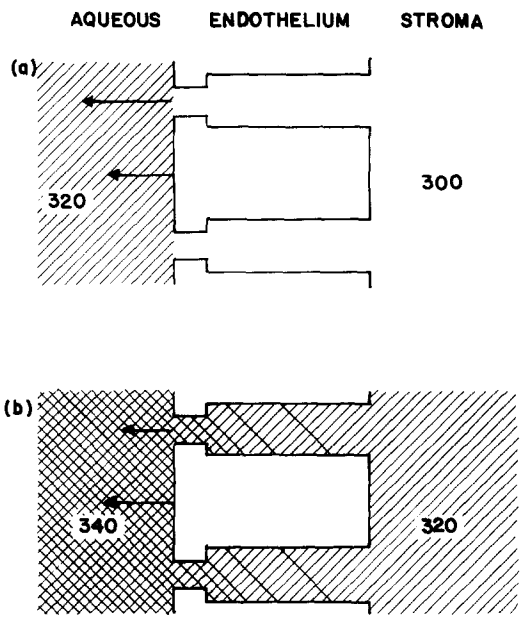

Fig. 3. A schematic illustration of the effects proposed to explain the variation of osmotic permeabilities. In (a), 20 mosM sucrose has been added to the 300 mosM aqueous solution, while no addition has been made to the 300 mosM stroma solution. In (b), 40 mosM sucrose has been added to the aqueous and 20 mosM sucrose to the stromal solution. Here, the fluid flow sweeps sucrose into the intercellular channel. This reduces the concentration differences that drive the osmotically induced fluid flows across both the tight junction and the apical cell membrane. Hence, for the same 20 mosM concentration difference, the arrows, proportioned to the predicted flows, show that there is less fluid flow in (b) than in (a).

In the second series of experiments, sucrose was added to the stromal solution so as to induce osmotic flow from aqueous to stroma. Sucrose in the stroma would be able to diffuse into the intercellular channel through its open stromal end, but the fluid flow would tend to convect sucrose out of the channel. Thus, in the steady state, there would be a standing gradient of sucrose within the extracellular channel. As shown in Fig. 4, for such case one would expect only little change in the measured water permeability

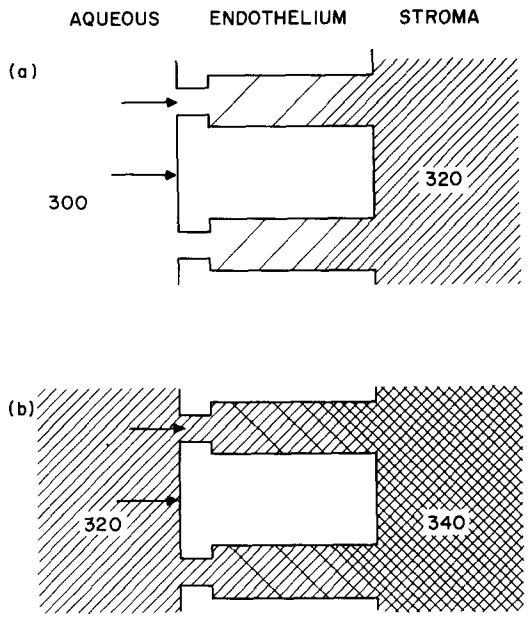

Fig. 4. The solutions bathing the corneal endothelium in Fig. 3 have been interchanged here so that the stroma has now been made 20 mosM hypertonic to the aqueous. Note that in both cases shown here sucrose is swept out of the intercellular channel; hence, the reduction in the predicted fluid flow here from (a) to (b) is much smaller than that shown in Fig 3.

with increasing stromal sucrose concentrations. This is because the change in the measured P_{os} depends upon the concentration gradient created as sucrose is swept from the stroma into (or out of) the intercellular channel. In the first series of experiments, raising the sucrose concentration on both sides should produce a significant change in P_{os} because there was previously no sucrose on the stromal side. However, in the second series of experiments, raising the sucrose concentration on both sides only increments the already present sucrose on the stromal side and so should produce a small change in P_{os}. Once more, in agreement with our expectation we found no detectable change ($P_{os} = 28 \pm 4$ μm/s versus $P_{os} = 31 \pm 5$ μm/s) when the stromal sucrose concentration was increased by 20 mM.

B. Effects of unstirred layers

One has to consider the possibility that the decrease in P_{os} with increased bathing solution concentration that we measured could be an artifact caused by unstirred layers outside the cell layer. Of course, it should be noted that, in our interpretation, the effect observed is itself the result of an unstirred layer, not one broadside against the aqueous or stromal faces of the tissue, but rather one trapped within the intercellular channel between the cells.

We can attempt to quantify the expected magnitude of the 'sweeping-in' effect by using a recent treatment of unstirred layers [4,10] which showed that in the connective tissue supporting an epithelium, such as the stromal side of the corneal endothelium, the significance of the unstirred layer can be estimated from a parameter β which is equal to the convective solute velocity divided by the diffusional solute velocity. Of course, the usual theory of unstirred layers can be only crudely applied to an 'unstirred layer' trapped within the intercellular channel. Nonetheless, it is revealing to calculate the ratio

$$\frac{\beta(\text{channel})}{\beta(\text{stroma})} = \frac{[L(\text{channel})/\alpha(\text{channel})]}{[L(\text{stroma})/\alpha(\text{stroma})]}$$

where L is the length and α is the fraction of cross-sectional area available for fluid passage. Since $L(\text{channel}) \sim 10^{-3}$ cm, $L(\text{stroma}) \sim 3 \cdot 10^{-2}$ cm, $\alpha(\text{channel}) \sim 1/300$, and $\alpha(\text{stroma}) \sim 0.5$, this means that $\beta(\text{channel})/\beta(\text{stroma}) \sim 5$. Thus the effect of the 'unstirred layer' trapped within the intercellular channel would be expected to be more important than the unstirred layer in the stroma. In addition, an unstirred layer adjacent but exterior to the aqueous or stromal faces should decrease P_{os} with increasing solution concentration, but that effect should occur equally for osmotically induced flow towards the aqueous and towards the stroma. Thus, the asymmetric behavior we observed cannot be accounted for by the effect of unstirred layers exterior to the cell layer.

C. Priming of the fluid pump and time-course of the osmotic flow

After concentration gradients across the tissue were removed, we found that the fluid flow often changed less than what had been anticipated from the previous increase (see Fig. 2). Thus, osmotic gradients can 'prime' the endothelial fluid movement in either direction. A priming effect for fluid flow osmoti-

cally driven towards the aqueous has been recently reported [8]. The authors theorized that this effect might be related to morphological changes in the endothelial cell layer.

We also observed that the rate of fluid movement did not change immediately after the imposition of an osmotic gradient. We speculate that this 10–30 min delay after a solution substitution may be related to changes in cell volume. For an assumed cell membrane permeability value of 30 μm/s, the time scale for these changes ought to be approx. 10 min. However, other processes might also introduce transients on a similar time scale; for example, the stromal transients of concentration and pressure calculated in a theoretical model [11].

Acknowledgements

This work was supported by U.S.P.H.S. Research Grant EY-01080 and RCDA EY-00006 to J.F. L.S.L. was a U.S.P.H.S. Postdoctoral Trainee supported by Grants EY 07014 and EY 07002.

References

1 Mishima, S. and Kudo, T. (1967) Invest. Ophthal. 6, 329–339
2 Maurice, D.M. (1972) J. Physiol. (London) 221, 43–54
3 Ussing, H.H., Bindslev, N. Lassen, N.A. and Sten-Knudsen, O. (1980) Proc. 15th Alfred Benzon Symposium, Copenhagen, Munksgaard, Copenhagen
4 Pedley, T. and Fischbarg, J. (1980) J. Membrane Biol. 54, 89–102
5 Diamond, J.M. (1979) J. Membrane Biol. 51, 195–216
6 Dikstein, S. and Maurice, D.M. (1972) J. Physiol. (London) 221, 29–41
7 Fischbarg, J., Lim, J.J. and Bourguet, J. (1977) J. Membrane Biol. 35, 95–112
8 Fischbarg, J., Hofer, G. and Koatz, R. (1980) Biochim. Biophys. Acta 603, 198–206
9 Leibovitch, L.S. and Weinbaum, S. (1981) Biophys. J., in the press
10 Pedley, T. and Fischbarg, J. (1978) J. Theor. Biol. 70, 427–447
11 Klyce, S.D. and Russel, S.R. (1979) J. Physiol. 292, 107–134