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The osmotic permeability of isolated calf pulmonary artery endothelial cells

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Osmotic permeability coefficients, P_F, for water in isolated calf pulmonary artery endothelial cells determined over the temperature range 41 to 20 °C are $311 \cdot 10^{-5}$ cm · s^{-1} at 37 °C and $159 \cdot 10^{-5}$ cm · s^{-1} at 20 °C. The value at 37 °C is close to that reported earlier for the diffusional permeability coefficient, P_D. The P_F/P_D ratio is 1.0 at 37 °C. The P_F values are within the range of values extrapolated for filtration permeability in pulmonary endothelium. The temperature dependence expressed as the activation energy is 7.2 kcal · mol^{-1}. The product of hydraulic conductivity, L_p (or P_F) and of viscosity changes in water is not constant from 37 to 20 °C. These results can be interpreted to indicate a similar pathway for water whether under diffusional or osmotic gradients.

Introduction

Water is ubiquitous in biological systems and regulation of water movement into and out of cells is of critical importance to the maintenance of normal cell function. Movement of water through some cells, especially endothelial and epithelial cells, also has a potential role in regulating water movement between body compartments. The barrier properties of the endothelial cells to water, as a part of the endothelium, have been a topic of controversy for some time. Some investigators consider the role of the endothelial cells to be negligible while others consider it to be substantial in different organs, at different times [1–3].

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In all of these studies, whether in whole animals or isolated capillaries, one has to deduce the cell permeability under conditions where both the cell and the extracellular pathway are potential routes for solute movement. We have approached this problem by using isolated endothelial cells where the cell permeability can be measured independently of the contribution from the extracellular pathway. Resolution of the question of the regulation of endothelial cell water permeability will provide information of importance both from a basic physiological viewpoint and from a clinical perspective.

We have been studying the characteristics of the endothelial cell that are related to the movement of water across the plasma membrane. We have reported the diffusional permeability coefficient at 37 °C for tritiated water (^{3}HHO) to be $304 \cdot 10^{-5}$ cm · s^{-1} in calf pulmonary artery endothelial cells [1]. The values reported for water is in

the range that accords with studies of endothelial diffusional permeability in intact lungs [1,2]. We now have measured the permeability of isolated endothelial cells to water under an osmotic gradient over the temperature range from 41 to 20 °C. These studies combined with our earlier results allow us: (1) to compare the diffusional and osmotic permeability to water; (2) to estimate the temperature dependence of osmotic permeability; and (3) to compare these results to those in intact lungs. We can, for the first time, define the hydraulic conductivity (L_p) and the osmotic permeability coefficient (P_F or P_{os}) for isolated endothelial cells and suggest, on the basis of experimental data, the pathway used by water to enter the endothelial cell under an osmotic gradient.

Materials and Methods

Cell preparation

Endothelial cells are isolated from calf pulmonary artery and maintained and passed in culture by means of nonenzymatic techniques [4]. The cell surface of the glycocalyx is left intact with this technique. The cells are characterized as being of endothelial origin by testing for Factor VIII antigen and for angiotensin-converting enzyme activity and by morphological criteria under light and electron microscopy [5]. For the osmotic studies the medium is removed from the T-75 flasks; 5 ml of Puck's A saline (in g/l: KCl 0.40; NaCl 8.00; $NaHCO_3$ 0.35; D-glucose 1.00; phenol red 0.005 (Gibco)) with 20 mM Hepes and 0.05% EDTA (Puck's AI) pH 7.4 at 4 °C are then added to the flasks. After 5 min the flask is scraped with a rubber policeman and the cells are transferred to a centrifuge tube. The cells are centrifuged at $270 \times g$ for 10 min at 4 °C. The supernatant fluid is removed and the cells are resuspended in 1.0 ml of Puck's A with 20 mM Hepes (adjusted to 320 mosM with NaCl) (Puck's AII) by trituration with a 22 gauge needle. The cells are centrifuged for 5 s in a Microfuge B (Beckman). The cells are resuspended in 1 ml of Puck's AII and triturated with 22 gauge and 26 gauge needles. A sample of cells is removed, stained and counted with erythrosin B in a hemocytometer and viability is assessed. Cell volume and water content are measured as described previously [6].

Measurement of cell volume changes

Cell volume changes as a function of time are monitored as changes in percent transmittance due to changes in light scattering, the basis for which has been described by others [7,8]. In our studies, the change in transmittance is monitored with a Beckman DB-GT spectrophotometer with a full-scale deflection (200 mV) corresponding to a 10% change in transmittance at 592 nm which is recorded from the millivolt output signal of the instrument. The change in signal is amplified and recorded digitally and graphically.

2 ml of cells in Puck's AII, approx. $(2–3) \cdot 10^6$ cells/ml, are added to the sample cuvette and Puck's AII to the reference cuvette; both are maintained at temperature in a thermojacketed cuvette compartment. Rotary stirrers (Spectrocell) are placed in both cuvettes to insure adequate mixing and to prevent unstirred layers and cell settling. A 100 μl bolus of an NaCl solution is added to the cuvette and the volume change recorded. The time for mixing to be complete (0.6 s) and the response time of the instrument and recording system (0.1 s) are rapid enough to prevent interference with monitoring of the cell response. An example of the tracings is given in Fig. 1.

Transmittance change as a function of time is recorded in units proportional to the millivolt output. Millivolt readings are converted to volume changes by comparing the linear relation for the change in millivolts vs. 1/osmolality to the cell

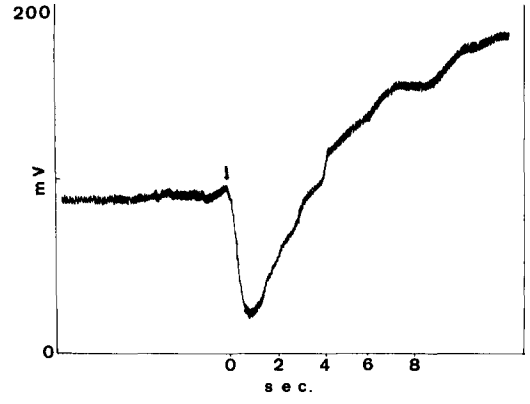

Fig. 1. A tracing of the change in millivolt readings as a reflection of the change in percent transmission with the addition of 100 μl of 1.5 M NaCl to endothelial cells suspended in Puck's AII. The arrow indicates the injection.

volume vs. 1/osmolality and calculating a constant which relates the change in millivolts to the change in cell volume. Examples of each of these are given in Fig. 2. The final osmolality of the cell medium is obtained from freezing point depression (Osmette) after removal of the cells by centrifugation in a Microfuge B.

The hydraulic conductivily is calculated as described previously [6] with Hempling's integration [7] of Lucké's equation ($dv/dt = L_p \cdot SRT\Delta c$). P_F is calculated from the relation $P_F = L_p \cdot RT/\bar{V}_w$. S is surface area, R is the gas constant, T is the absolute temperature, Δc is the change in concentration and $\bar{V}_w$ is the partial molar volume of water. In Hempling's solution the volume rather than surface area of the cells is used; this obviates the uncertainty inherent in calculations which require estimates of surface area.

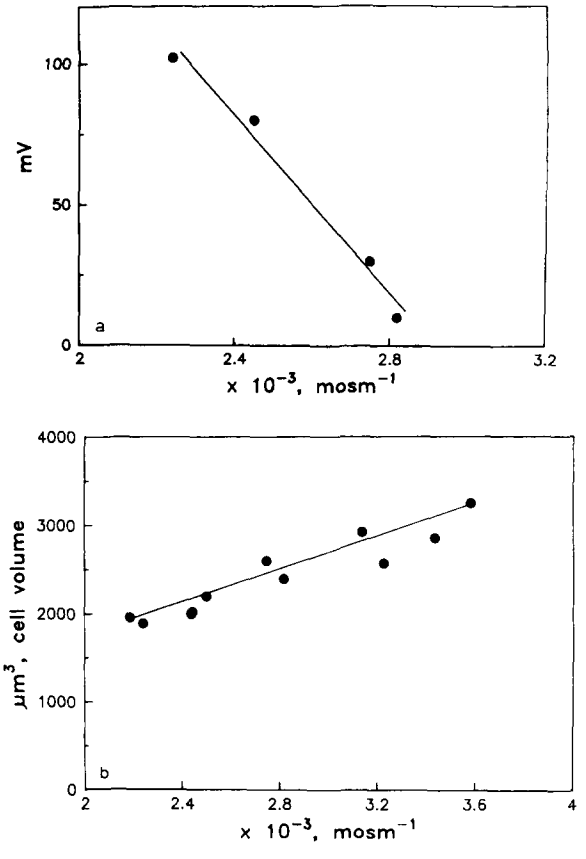

Fig. 2. (a) An example of the change in millivolts against the reciprocal of osmolality. Linearity is established by analysis of variance with F for regression of 103. (b) Cell volume is plotted against the reciprocal of osmolality. Linearity is established by analysis of variance with F for regression of 52.37.

Results and Discussion

The endothelial cells have a water volume (^{3}HHO) of 2925 ± 70 μm^3 at 320 mosmol and a fractional water content of 0.74 (w/w). The cell diameter at 320 mosmol is 21.00 ± 0.52 μm measured by microscopy and cell density is 1.042 g/cm^2 determined by centrifugation in Percoll (Pharmacia). A linear relation is seen between cell volume and the reciprocal of osmolality in Fig. 2b. Over the range of osmolality tested (279–562 mosM) the cell volume changed from 1890 μm^3 to 3250 μm^3. Ponder's R, which is considered an indication of the fraction of the total cell water taking part in an osmotic response, is 0.86. The b value [7] calculated graphically is 350 μm^3.

Measurement of L_p and P_F

The values of L_p calculated at 41, 37, 30 and 20 °C for the endothelial cells are listed in Table I and range from $25.5 \cdot 10^{-10}$ to $11.5 \cdot 10^{-10}$ cm · (cm $H_2O \cdot s)^{-1}$. The values of P_F (or P_{os}), calculated from these values of L_p, range from $377 \cdot 10^{-5}$ to $159 \cdot 10^{-5}$ cm · s^{-1} and are given in Table I. These values are calculated over the first 3–5 s after addition of the osmotic bolus. An example of the plot of the Hempling equation is given in Fig. 3. The slope, which is the hydraulic conductivity, is calculated by analysis of variance. Values for other cells at 20 and 37 °C are listed in Table II. Excluding the erythrocyte, the values of L_p range from 5.9 to 27 at 20 °C and from 19.1 to 67 at

TABLE I

L_p AND P_F AT DIFFERENT TEMPERATURES FOR ENDOTHELIAL CELLS

Values are given as the mean ± S.E.; the number of determinations is in parenthesis.

Temp (°C)	$10^{10} L_p$ (cm·(cm $H_2O \cdot s)^{-1}$)	$10^5 P_F$ (cm·s^{-1})
41	25.5 ± 1.1 (7)	377 ± 16 (7)
37	23.0 ± 2.5 (6)	311 ± 37 (6)
30	16.2 ± 1.7 (10)	239 ± 25 (10)
20	11.5 ± 0.7 (5)	159 ± 10 (5)

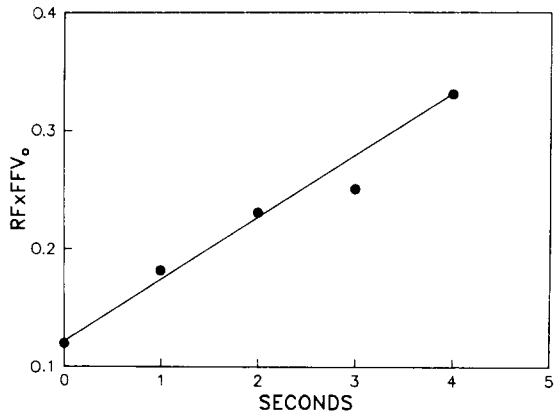

Fig. 3. $RF \times FFV_0$ is plotted against time in seconds. $RF \times FFV_0$ is the solution of Hempling's equation and can be expressed as $RF \times FFV_0 = 1/[36^{1/3} P_0 V_0]\ f(VV_e)$ [6,7], where the subscript of zero indicates time = 0 and subscript e = equilibrium. The slope of this plot is k_{2a}, the permeability coefficient as defined by Hempling. L_p is calculated from k_{2a} by converting the units to cm·(cm H_2O·s)$^{-1}$. This plot is from one run and is an example of the data obtained. Linearity is established by analysis of variance with the slope equal to 0.032 and $F = 124.8$.

37 °C, all in units $\cdot 10^{-10}$ cm · (cmH_2O · s)$^{-1}$. The endothelial cell hydraulic conductivity falls within the lower to middle part of this range.

None of the other cells listed in Table II are from tissues which normally form a part of the lining of organs as do the endothelial cells. We are not aware of any reports of the measurement of the hydraulic conductivity for isolated cells from endothelium or epithelium, although there are many estimates of these in intact tissue layers (Refs. 9 (pp. 165, 296, 324), 10 and 11). The measurements of the endothelial or epithelial permeability in tissue layers generally produce values for epithelium which are much lower than those for the endothelium. The presence of much tighter intercellular junctions in the epithelium is usually cited as an explanation for the difference, with the implicit assumption that the transcellular pathway for water is minimal.

The values for P_F are within the range calculated for filtration permeabilities ($2.5 \cdot 10^{-5}$ to $3.6 \cdot 10^{-2}$ cm · s^{-1} at 37 °C) [3,12] in intact mammalian pulmonary endothelium. Measurements of L_p have also been made in isolated capillaries but these are in amphibia at 20–25 °C [10]. These values vary in different parts of the capillary but are approximately (700–800) $\cdot 10^{-10}$ cm · (cm H_2O · s)$^{-1}$. Bullfrog pulmonary epithelium, on the other hand, is reported to have an L_p of $472 \cdot 10^{-10}$ cm · (cm H_2O · s)$^{-1}$ at 25 °C [11]. The L_p values

TABLE II

L_p, P_F AND E_a FOR CELLS AT 20 AND 37 °C

	Temp. (°C)	$10^{10} L_p$ (cm·(cm H_2O·s)$^{-1}$)	$10^5 P_F$ (cm·s^{-1})	E_a (kcal·mol^{-1})
Endothelial	37	23	311	7.2
cells	20	11.5	159	
Alveolar macrophages [6]	20	15.6	217	
Novikoff	37	19.1	265	10.4
hepatoma [15]	20	5.9	82	
Ehrlich	37	67	980	10.0
ascites [7] [a]	20	25	363	
Pulmonary	37	65.8	954	9.9
fibroblasts [14]	20	27	375	
Erythrocytes [3,19]				
human	37	168	2335	6.95
	20	87.3	1214	
dog	37	221.5	3212	5.1
	20	142.9	1987	

[a] Over the years a range of values have been reported for the Ehrlich ascites cells; these are based on Fig. 4 in Ref. 7, where values for both 37 and 20 °C are plotted.

which we report for the endothelial cells are lower than those reported for most of the studies in endothelium. This difference between isolated cell permeability for most cell types and that for intact endothelium or epithelium has been noted by others although the comparisons are to nonendothelial cells.

Temperature dependence of water permeation

In order to evaluate the change in L_p (or P_F) which accompanies a change in temperature we have calculated the activation energy, E_a. We use the Arrhenius equation to evaluate the change in permeation with temperature and hence use the term activation. However, the permeation process is complex and cannot be analyzed on a molecular level from activation energies [8]. The E_a calculated for the L_p over the temperature range from 41 to 20 °C is 7.2 kcal · mol^{-1}. Comparison of permeability at different temperatures has been used to compare temperature dependence of the permeation process in different systems.

The values of E_a reported for other cells are listed in Table II. The values for the erythrocytes are 5–7 kcal · mol^{-1} [9,13] and those for other cells 9–10 kcal · mol^{-1} [14,15]. Measurements of E_a in artificial membranes range from around 4.5 in a porous membrane to 12–15 kcal · mol^{-1} in a lipid membrane [9]. This difference has been used as a method to distinguish aqueous and lipid pathways [13]. Although there is much discussion about the validity of this interpretation [8,9], we can compare the value we obtain to that in other cells. Our value is higher than that usually associated with an aqueous pathway and lower than that usually identified with a lipid pathway. However, in other cells an activation energy close to 7 kcal · mol^{-1} is reported where there is other evidence for a lipid pathway for water (e.g., Ref. 15).

Viscosity changes with temperature

The changes in the viscosity of water with a change in temperature can contribute to the temperature dependence of P_F if water movement is through aqueous pathways. Viera et al. [13] calculated the product of the viscosity of water (η_w) and L_p of erythrocytes at different temperatures. The product remained constant for the erythrocytes and Viera et al. interpreted this as evidence of viscous flow through aqueous pores in the membrane. This approach has been used in other cells [15] and in capillary endothelium [10] to demonstrate the presence [10] or absence [15] of viscous flow. In Table III the products of η_w and L_p for the endothelial cells at 20, 30, 37 and 41 °C are listed. We interpret the lack of constancy of the product as evidence that water movement does not have the characteristics of viscous flow in the endothelial cells.

TABLE III

PRODUCT OF L_p FOR ENDOTHELIAL CELLS AND THE VISCOSITY OF WATER (η_w) [20] AT DIFFERENT TEMPERATURES

°C	η_w (cP)	$L_p \cdot \eta_w$
41	0.64	15.8
37	0.69	15.9
30	0.80	10.7
20	1.00	11.5

We reported a value of $304 \cdot 10^{-5}$ cm · s^{-1} for P_D at 37 °C [1] and in a preliminary report $160 \cdot 10^{-5}$ cm · s^{-1} at 20 °C [16] for the endothelial cells. From this we calculate a P_F/P_D ratio of 1.0 at 37 and 20 °C for the endothelial cells. A criterion for the distinction between convective and diffusional transport is the relationship of P_F to P_D as stated by Lassen and Perl [17] (based on Hevesy, Hofer and Krogh [21]): if the movement is convective, $P_F \gg P_D$, and if it is diffusional, $P_F = P_D$. If we accept the criterion just stated we must conclude that a major portion of the movement across the plasma membrane of the endothelial cell is by a diffusional process under osmotic driving forces as well as diffusional driving forces. A similar condition has been suggested for erythrocyte (e.g., Refs. 8 and 18) and Ehrlich ascites cells [7].

Summary

The calf pulmonary artery endothelial cell isolated and cultured without the use of proteolytic enzymes is used in these studies. At the present time there are no data to indicate that permeation of small molecules into these cells differs from endothelial cells in the microcirculation, so they provide a starting point for these studies. The

hydraulic conductivity and permeability coefficient for water movement into these cells over the temperature range from 20 to 41 °C are within the range that would be predicted in pulmonary endothelium based on whole organ studies, although this range is quite wide. The endothelial cell values which we report are lower than those reported in isolated frog capillary endothelium at 25 °C. Compared to other cells the coefficients in the endothelial cells are in the low to middle part of the reported range. If we consider all of these comparisons, the measurement of the endothelial cell permeability in this system provides a method for estimating the role of the endothelial cell in the endothelial barrier.

The combination of (1) the characteristics of the temperature dependence of permeation, (2) the comparison to diffusional water movement at 37 and 20 °C and (3) the lack of constancy of $L_p \cdot \eta_w$ prompts us to propose that water movement through the endothelial cell membrane is by diffusion through lipid areas. Determination of the temperature dependence of diffusional permeation should help to confirm or refute this hypothesis.

Acknowledgments

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