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PERMEABILITY PROPERTIES OF THE SUBEPITHELIAL TISSUES OF *NECTURUS* GALLBLADDER

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The permeability properties of the subepithelial connective tissue of *Necturus* gallbladder were evaluated by measurement of electrical resistance, dilution potentials and hydraulic water permeability. The gallbladder epithelial cells were removed by scraping and the underlying connective tissue placed in an Ussing chamber. The electrical resistance was $2.2 \pm 0.8\ \Omega \cdot cm^2$; the tissue was slightly cation selective relative to free solution. The subepithelial tissues restricted the rate of diffusion of small solutes to 50% of the free solution value. The hydraulic water permeability averaged $2.1 \cdot 10^{-2}$ cm/s per atm. We conclude that limitations of the area of subepithelium available for fluid movement are the most important factors in determining the restrictions to solute and water flow offered by the subepithelial tissues.

Introduction

The solute and water permeabilities of the subepithelial basement membrane and connective tissue are significant factors in most models of fluid transporting epithelial tissue such as gallbladder, intestine and renal proximal tubule. Mathematical models of epithelia suggest that the rate of solute and water movement through the subepithelial layers is reduced by the basement membrane and connective tissue which lies between the epithelium and the capillaries [1,2]. Few measurements of the properties of the subepithelial connective tissue layers have been made. The most frequently cited of these papers is the work of Welling and Grantham [3] who measured the hydraulic water permeability (L_p) of the basement membrane of rabbit renal proximal tubule. These authors also demonstrated that the basement membrane did not reflect small solutes and that only albumin was significantly restricted in its movement across the basement membrane. Recent mathematical models of fluid transport by *Necturus* gallbladder epithelium indicate that the restriction to solute diffusion offered by the subepithelial tissues determines the magnitude of the transepithelial osmotic gradient required to stop fluid transport [1,2]. In our studies of water permeability of the cell membranes of *Necturus* gallbladder epithelium [4] we concluded that the epithelial cells were effectively isolated from the serosal bath by the unstirred layer formed by the subepithelial connective tissue and basement membrane. This conclusion was based on the observation that the epithelial cells behaved as osmometers with respect to the mucosal bath [4].

In the present paper we measured the electrical resistance, dilution potentials and hydraulic water permeability of isolated subepithelial tissue from *Necturus* gallbladder. We show that this connective tissue layer offers limited resistance to solute and

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water movement and is slightly cation selective when compared to free solution.

Materials and Methods

Adult *Necturus* were stored and anesthetized as previously described [4]. Gallbladders were excised, rinsed free of bile, and the epithelial cells were removed by gentle scraping with the edge of a glass slide. The epithelial cells were readily separated from the underlying connective tissue. The subepithelial tissue was supported on a nylon mesh in an Ussing chamber and perfused with *Necturus*-Ringer solution with the following composition: 100 mM Na, 98 mM Cl, 2.5 mM K, 1.8 mM Ca, 1.0 mM Mg, 10 mM HCO_3, 0.5 mM PO_4. The solutions were gassed continuously with 99% air/1% CO_2 to maintain a pH of 7.6. A ten-fold dilution of one bathing solution was used in the determination of dilution potentials.

Electrical connections were achieved by calomel half cells connected through Ringer-filled channels to the chamber halves. The mucosal bath was connected to ground. Current-passing electrodes were chlorided silver wires connected by Ringer agar bridges to the chamber. Voltages and currents were measured with multimeters (Model 2200A. J. Fluke Inc.) and recorded manually.

Hydraulic water permeability was calculated from the rate of volume flow across the tissue induced by a hydrostatic pressure gradient. Hydrostatic pressure gradients were created by elevation of a reservoir connected to one of the chamber halves. After each experiment the electrical resistance of the chamber and nylon mesh were measured and appropriate corrections made. The hydraulic resistance of the chamber and mesh, measured in preliminary experiments, were shown to be negligible compared to the tissue hydraulic resistance. The area of the tissue in each experiment equaled 0.28 cm^2. All data are presented as mean and standard error with *t*-tests for significance of differences.

Results

Electrical resistance. The electrical resistance of 16 subepithelial tissues were determined with the tissue bathed in control Ringer solution. Tissue resistance averaged $2.2 \pm 0.8\ \Omega \cdot cm^2$. The relatively large standard error was due in part to the low resistance of the tissue. The average tissue resistance was $7.9 \pm 2.8\ \Omega$ and the resistance of the chamber and supporting mesh equaled $269 \pm 5.1\ \Omega$. The solution resistances thus far exceeded the tissue resistance and constituted a significant source of error because of undetected small air bubbles or debris trapped in the nylon mesh. The current-voltage relationship for the subepithelial tissues was linear over a range of ± 500 mV.

Dilution potentials. The mucosal or serosal bathing solution was replaced by Ringer diluted ten-fold and the resultant dilution potential measured. Corrections were made for small offsets in the calomel electrodes after each measurement. Dilution of the mucosal bath produced a negative voltage which was sustained as long as the solution asymmetry was maintained. The dilution potential obtained after correction for electrode offset was -6.2 ± 0.4 mV ($n = 10$). Dilution of the serosal bath produced a positive voltage of similar magnitude 6.1 ± 0.8 mV ($n = 9$). The symmetry of the dilution potentials permitted pooling of the data and the average dilution potentials equaled 6.2 ± 0.4 mV ($n = 19$) with the dilute side negative.

The dilution potential (ΔV) may be used to calculate the relative transference numbers of cations and anions, assuming that Na and Cl are the major current carring ions, that the activity of Na and Cl are approximately equal to one another in each bath, and that the tissue can be represented as a simple, single barrier. The expression which relates these parameters is [5]:

$$\Delta V = (t^+ - t^-)\, RT \ln \frac{[\mathrm{NaCl}]_1}{[\mathrm{NaCl}]_2} \qquad (1)$$

where t^+ is the cation transference number, t^- is the anion transference number, R and T have their usual meaning, and the [NaCl] terms refer to the magnitude of the concentration in the two baths. The transference numbers calculated from the average dilution potential of 6.2 mV were: t^+ 0.45, t^- 0.55. The transference numbers for Na and Cl in free solution are t_{Na} 0.33, t_{Cl} 0.67 [6]. Thus the subepithelial tissues slightly favor the movement of cations and retard that of anions relative to free solution.

Hydraulic water permeability. The water permeability of the subepithelial tissue from five gallbladders was determined from a total of 55 measurements of the water flow induced by hydrostatic pressure. Hydrostatic pressures from 15.5 to 117.5 cm H_2O were utilized. Hydraulic water permeability averaged $2.03 \pm 0.06 \cdot 10^{-5}$ cm/s per cm H_2O, or $2.1 \cdot 10^{-2}$ cm/sec per atm. The rate of water flow was a linear function of the hydrostatic pressure difference across the subepithelial tissues with no evidence of non-linearity or rectification of flow.

Subepithelial tissue dimensions and solution osmolality. The subepithelial tissues of three gallbladders were observed in a light microscope equipped with differential interference contrast optics. The thickness of the tissues was estimated from the focal displacements required to visualize the upper and lower tissue surfaces. The microscope stage displacements were calibrated with a micrometer and the fine focus knob was driven by a stepping motor as previously described [4]. Tissue thickness measurements were corrected for refractive index differences. Steady-state tissue thickness was measured in control Ringer, in Ringer diluted ten-fold (osmolality about 20 mosM), and in Ringer to which mannitol had been added to increase the osmolality to 600 mosM. The results of these experiments are given in Table I. Tissue thickness was not significantly altered by exposure to hypertonic and hypotonic media.

Discussion

The subepithelial tissues of *Necturus* gallbladder slightly restrict the movements of solutes and water. The limitation to the movement of solutes presumably arises because of restricted diffusion through the basement membrane and connective tissue. The diffusion coefficient (D) for NaCl in the subepithelial tissues may be estimated from the electrical conductance (G) and tissue thickness (Δx) as follows [5]:

$$D = \frac{RT\Delta xG}{CF^2} \qquad (2)$$

where F, R and T have their usual meaning, and C is the NaCl concentration. The resultant diffusion coefficient equals $7.8 \cdot 10^{-6}$ cm^2/s. The equivalent NaCl permeability of the supporting tissue is $1.2 \cdot 10^{-3}$ cm/s or about 100-times that of the epithelium. The subepithelial tissues effectively restrict the diffusion of NaCl to one-half of its free solution value of $1.5 \cdot 10^{-5}$ cm^2/s [6]. The hydraulic water permeability of the subepithelial tissue of *Necturus* gallbladder was $2.1 \cdot 10^{-2}$ cm/s per atm which is about 500-times greater than that of the epithelial cells [4]. The thickness of the subepithelial tissue as well as the area of tissue available for solute and water movement should be the primary determinants of the magnitude of the limitation to diffusion or filtration offered by these tissues.

Table I shows that *Necturus* gallbladder subepithelial tissues are about 65 μm thick. In other preparations the submucosa is considerably thicker and would be expected to constitute a more significant barrier. Rabbit gallbladder subepithelial tissue is about 300 μm thick and offers an electrical resistance about 5-times that of *Necturus* submucosa [7]. Removal of the epithelium from the gallbladders of a variety of animals has been utilized to obtain estimates of the electrical resistance of the subepithelial tissues. Gelarden and Rose [8] obtained resistances of the subepithelial tissues from a number of different species ranging from 6 to 26 $\Omega \cdot$ cm^2. The subepithelial tissues contributed about 25% of the total electrical resistance of these gallbladders. Henin et al. [9] made microelectrode measurements in gallbladders of fish, amphibia, birds and mammals and found subepithelial tissue resistance ranging from 5.9 to 23.1 $\omega \cdot$ cm^2. Again the subepithelial tissue resistance amounted to 10–40% of the total tissue

TABLE I

SUBEPITHELIAL TISSUE DIMENSIONS AND SOLUTION OSMOLALITY

Normal Ringer osmolality was 200 mosmol. All differences not significant by paired t-test.

Solution osmolality (mosmol)	Thickness (μm)
200	65.3 ± 6.4(3)
20	72.7 ± 4.6(3)
600	53.3 ± 1.8(3)

resistance. Wright and Diamond [7] estimated that removal of the epithelium from rabbit gallbladder by scraping and chloroform treatment reduced the electrical resistance by 94%. An estimate of the resistance to diffusion of small solutes in fish gallbladder subepithelial tissues was made by Diamond [2]. He calculated that solute diffusion was restricted by a factor of ten in the connective tissues presumably because of a reduction in mean free path.

Further limitations to solute movement across the subepithelial layers arise because of reductions in the area across which solute movement occurs. If the exit of solute from the epithelium is primarily confined to the lateral intercellular spaces, the diffusional resistance of the subepithelial tissues then becomes a complex function of the geometry of the pathways of solute movement across the epithelium. Weinstein et al. [2] estimated from their mathematical model of rabbit gallbladder that the subepithelial tissues should offer a 16 $\Omega \cdot cm^2$ resistance. Their calculations suggested that area restrictions as well as diffusion limitations combined to produce a four-fold reduction in the permeability of the subepithelial tissue compared to an unstirred layer of equivalent thickness. Weinstein et al. [2] also developed a theoretical relation between the magnitude of the osmotic gradient required to stop fluid transport, $\Delta\pi$, the active solute transport rate, J_a, and the solute permeability of the subepithelial tissues, P_s. The expression which relates these parameters is:

$$P_s = J_a/\Delta\pi \qquad (3)$$

In *Necturus* gallbladder, $\Delta\pi$ is about 20 mosM [4], and J_a is estimated to be $350–680 \cdot 10^{-12}$ osM/cm^2 per s [11]. P_s is then calculated to range from $1.75 \cdot 10^{-5}$ to $3.4 \cdot 10^{-5}$ cm/s or about 35 to 70 times less than the measured values of $1.2 \cdot 10^{-3}$ cm/s. These calculations suggest that the area of subepithelial tissue available for solute diffusion from the epithelium is reduced by a factor of 35 to 70 in *Necturus* gallbladder.

The hydraulic water permeability of the subepithelial tissues may also be used to obtain an approximation of the reduction in area available for solute and water movement. The fluid transport rates measured in sac preparations of *Necturus* gallbladder range from $1.6 \cdot 10^{-6}$ cm/s to $3.4 \cdot 10^{-6}$ cm/s [11]. The hydrostatic pressure required to drive the transported fluid across the subepithelial layers would then be approximately 0.1 to 0.2 cm H_2O. This is considerably less than a previous estimate of 3 cm H_2O [12]. This estimate was obtained by measuring the transepithelial hydrostatic pressure required to dilate the lateral intercellular spaces in a non-transporting epithelium [12]. Comparison of these results suggests that fluid movement across the subepithelial tissues may be restricted 15 to 30 times by a reduction in the available area of the subepithelium. The most important effects of limitations to water flow across the subepithelial tissues are probably due to hydrostatic pressures developed between the epithelium and these underlying structures. Small hydrostatic pressures alter *Necturus* gallbladder epithelial interspace and cell geometry because of the high compliance of the cell membranes [12]. Van Os [13] studied the hydraulic water permeability of the subepithelial connective tissues of rabbit gallbladder and reported that the steady-state flow rate in response to hydrostatic pressure was about five times greater than that of the intact preparation. We would thus conclude that the most significant restriction to fluid movement across the subepithelial layers probably arise not from the inherent properties of the subepithelial tissues but from a limitation in the area of these tissues available for fluid movement.

Finally, the osmotic forces generated by the epithelium result in the hydrostatic pressures needed to move fluid out of the lateral intercellular spaces and across the subepithelial tissues. The magnitude of the hydrostatic pressure required is directly determined by the Lp of the subepithelial tissues. Maximal stimulation of transport leads to dilatation of the lateral intercellular spaces and, in the extreme, to subepithelial blisters [14] because of the restriction to flow offered by the supporting tissues. Thus under these extreme conditions it is possible to demonstrate that the subepithelial connective tissue constitutes a significant barrier between the epithelium and the serosal bathing solution.

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