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Water permeabilities and salt reflection coefficients of luminal, basolateral and intracellular membrane vesicles isolated from rabbit kidney proximal tubule

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The mechanisms of water transport across the rabbit renal proximal convoluted tubule were approached by measuring osmotic permeabilities and solute reflection coefficients of the brush-border and the basolateral membranes. Plasma and intracellular membrane vesicles were isolated from rabbit renal cortex by centrifugation on a Percoll gradient. Three major turbidity bands were obtained: a fraction of purified basolateral membranes (BLMV), the two others being brush-border (BBMV) and endoplasmic reticulum (ERMV) membrane vesicles. The osmotic permeability (P_f) of the three types of vesicle was measured using stop-flow techniques and their geometry was determined by quasi-elastic light scattering. P_f was equal to 123 ± 8 μm/s ($n = 10$) for BBMV, 166 ± 10 μm/s ($n = 10$) for BLMV and 156 ± 9 μm/s ($n = 4$) for ERMV ($T = 26$ °C). A transcellular water permeability, per unit of apical surface area, of 71 μm/s was calculated considering that the luminal and the basolateral membranes act as two conductances in series. This value is in close agreement, after appropriate normalizations, with previously reported transepithelial water permeabilities obtained using in vitro microperfusion techniques thus supporting the hypothesis of a predominantly transcellular route for water flow across rabbit proximal convoluted tubule. The addition of 0.4 mM $HgCl_2$, a sulfhydryl reagent, decreased P_f about 60% in the three types of membrane providing evidence for the existence of proteic pathways. NaCl and KCl reflection coefficients were measured and found to be close to one for plasma and intracellular membranes suggesting that the water channels are not shared by salts.

Introduction

Of all the water that is filtered by the kidney, two thirds are reabsorbed along the proximal tubule. This transport is nearly isoosmotic and the slight local hypotonicity or hypertonicity responsible for this water movement implies that tubular walls have very high water permeabilities. During the past two decades, microperfusion of renal tubules has been used, in rabbits, to define this transepithelial water permeability. Results became progressively more accurate as new parameters were taken into account, as for example the perfusion rate dependence of volume absorption, the presence of unstirred layers and the influence of the amplitude of the osmotic gradient. These studies gave results lying mainly between 1000 and 3000 μm/s for proximal convoluted tubule [1–3], when expressed by unit of apparent apical tubular surface. But these data represent a combination of transcellular and paracellular water permeabilities.

Recent measurements of the luminal and basolateral membrane water permeabilities gave experimental evidence of a transcellular water route. The first reported values were obtained for basolateral membranes by rapid video recording techniques on lumen-collapsed proximal convoluted tubules [4,5]. They were sufficiently high to account for the transepithelial permeability and there was some evidence of the existence of proteic water channels in these structures [6]. As in other epithelia, however, the apical cell membrane could be the rate-limiting barrier to transcellular osmosis.

The next step was to measure osmotic permeabilities of the luminal membrane. This was carried out on isolated brush-border membrane vesicles using light scattering techniques. Again, extremely high water permeabilities were found mediated by water proteic channels [7,8]. However, because luminal and basolateral water permeabilities obtained with the same experimen-

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tal approach are scarce, a direct comparison between the two membranes was difficult. The few existing data that were obtained by light scattering are still not satisfactory for a relevant comparison of the two plasma membranes for the following reasons. First, several studies revealed that the method of membrane isolation influenced the permeability and conductance values recorded [9–12]. Thus, when brush-border and basolateral membranes are isolated by different methods and from different animals, comparisons may not be significant. Second, it was suspected that plasma membrane preparations were contaminated by intracellular organelles [8]. As intracellular membranes may have permeabilities that are very different from that of the plasma membrane studied, the light scattering measurements could be misleading. Third, the size of the vesicles was not always precisely measured which is of importance as the volume/surface area ratio of the vesicles is involved in the calculation of P_f.

In addition to water permeability measurements, solute-reflection coefficients have been measured in these membranes to point out the possible interactions between salts and the identified water channels. In the rabbit proximal convoluted tubule perfused in vitro it had previously been found that water does not drag a significant amount of solute across the epithelium indicating a solute reflection coefficient of one [13,14]. To account for this, at least one of the plasma membranes, luminal or basolateral, must have water selective channels. Vesicle studies have demonstrated reflection coefficients of one for NaCl and KCl in rat brush-border membranes [15] whereas on lumen-collapsed rabbit tubules NaCl reflection coefficient values of the basolateral membrane varied from 0.5 [16] to 1 [5].

To gain insight in the mechanisms of water transport in the proximal convoluted tubule and to overcome some of the above mentioned limitations, we decided to compare water permeability values and reflection coefficients of the brush-border and the basolateral membranes all measured on vesicular systems with the same techniques. Brush-border (BBMV) and basolateral (BLMV) membranes were isolated simultaneously from the same rabbit and were then exposed to identical conditions: vesicle geometry was determined by quasi-elastic light scattering and water permeability coefficients by stop-flow light scattering. To evaluate the effects of cross-contamination by intracellular membranes, we adapted the isolation technique to obtain at the same time as the BBMV and BLMV samples, a fraction enriched in endoplasmic reticulum on which permeability measurements were also performed. We then took advantage of our preparation method to also measure NaCl and KCl reflection coefficients on brush-border, basolateral and intracellular membrane vesicles.

Methods

Vesicle preparation

Vesicles were prepared from 1.5–2 kg New Zealand male white rabbits. Brush-border (BBMV), basolateral (BLMV) and a third batch of intracellular membranes were simultaneously isolated from the renal cortex according to the method described by Boumendil-Podevin and Podevin [17]. Briefly, the rabbits were anesthetized with pentobarbital, the kidneys were then rapidly removed and rinsed in an ice-cooled buffer (250 mM sucrose, 0.1 mM phenylmethylsulfonyl fluoride, 2 mM Tris-Hepes, pH 7.4) in order to remove blood. Thin slices of renal cortex were chopped with scalpels, homogenized with a Dounce homogenizer for five strokes, then in a Teflon-glass homogenizer (10 strokes at 1000 rpm) and centrifuged at $1000 \times g$ for 10 min to eliminate the debris ($T = 4°C$ during all centrifugations). The supernatant was centrifuged at $22000 \times g$ for 30 min to collect the remaining membranes. The white fluffy upper layer of this pellet was homogenized in 16 ml of buffer to which 2 ml of 100% Percoll had been added. After a 45 min centrifugation at $40000 \times g$ with a fixed angle rotor, four major bands of turbidity were obtained on the Percoll gradient: the basolateral membranes formed the upper band, the second identifiable band was of brush-border membranes and the third band that of intracellular membranes. The three types of membrane were separately homogenized in 10 ml of 20 mM mannitol, 20 mM KCl, 2 mM Tris-Hepes (pH 7.4) and centrifuged for 15 min at $60000 \times g$ in order to eliminate the Percoll, which formed a glassy pellet on the sloping wall of the tube. The final membrane fractions were suspended in a buffer which was used for all the downhill experiments (20 mM mannitol, 2 mM Tris-Hepes, pH 7.4) at a protein concentration of 0.4 mg/ml. Proteins were determined by the method of Bradford [18] with bovine albumin as standard.

Vesicles were prepared in the morning and the stop-flow experiments performed in the afternoon of the same day. This procedure ensures against possible damage by long isolation procedures or freezing.

Enzyme assays

In each experiment activities of specific marker enzymes of cell membranes and intracellular organelles were measured on aliquots of the three vesicle batches and in the homogenate from which the vesicles originated. Maltase was measured as described by Dahlquist [19], Na^+/K^+-ATPase as described previously [17] and glucose-6-phosphatase as described by Harper [20]; succinate dehydrogenase (assayed according to Pennington [21] modified as described in Ref. 22) was used to evaluate cross contamination by mitochondrial membranes.

Vesicle size determination by quasi-elastic light scattering (QELS)

In a comparative study, Perevucnik et al. [23] showed that the size analysis of BBMV from rabbit small intestine by quasi-elastic light scattering led to values similar to those obtained by gel filtration on Sephacryl S-1000 or electron microscopy. But glutaraldehyde fixation, high speed centrifugation under vacuum and freezing which are necessary for electron microscopy size determination may influence vesicle size and shape and the effect could vary from one type of membrane to another. Because of its nonperturbing quality QELS was chosen for size analysis. Apart from being satisfactory for comparing membrane sizes, this technique is very economic in biological material enabling both stop-flow experiments and QELS measurements to be carried out on vesicles from a single preparation.

Theory. QELS [24] is a non-perturbing technique used to determine the diameter of particles ranging from 0.01 to 1 μm. This technique takes avantage of the Brownian motion of particles. In our suspension the vesicles moved randomly as a result of collisions with the molecules of the suspending medium, the motion depending on vesicle size, temperature and viscosity of medium. Measurement of the temporal fluctuations in the intensity of light scattered by the Brownian motion leads to the translational diffusion coefficient D of the vesicles. Then, assuming the vesicles to be spherical *, the Stoke-Einstein equation relates these four variables and enables the mean hydrodynamic radius R_H to be calculated as:

$$R_H = \frac{kT}{\pi \eta D} \quad (1)$$

where k is the Boltzman constant, T the absolute temperature and η the viscosity of the solvent.

D is obtained by the cumulant analysis [25] of the autocorrelation function $C(\tau)$) ** of the scattered light intensity. In the present study, a second-order cumulant analysis was calculated to fit the autocorrelation functions with a precision of better than 1 part in 10^3.

Detailed reviews of the theory, instrumentation, data analysis and applications of this technique have already been published (Pecora [26], Chu [27], Cummings and Pike [28].

* Even if all vesicles were oblate spheroids (having a minor axis equal to 25% of the major axis) an error of 50% in the volum vs. surface ratio would not be exceeded.

** The autocorrelation function is defined as follows:

$$C(t) = \langle I(0)I(\tau)\rangle = \lim_{T\to\infty} 1/T \int I(t)I(t-\tau)\,dt \quad (2)$$

where I is the scattered light intensity, T represents the total experimental duration over which the product of the intensity with delayed versions of itself is averaged.

Apparatus and method. Before each experiment, all solutions were carefuly filtered to remove dust and pipetted into alcohol-washed cylindrical glass scattering cells. Measurements were made at 10°C to prevent vesicle damage during the experiment. The viscosity of the buffer (20 mM mannitol, 2 mM Tris-Hepes, pH 7.4) measured with a Schott Geräte viscosimeter was 1.3215 at 10°C.

The apparatus employed for QELS consisted of an Argon ion laser (Spectra Physics 164, $\lambda = 514.5$ nm), a temperature controlled scattering cell holder with toluene index matching bath, a Malvern autocorrelator linked to a Commodore computer for on line analysis. Measurements were performed at a scattering angle of 90°.

For each type of vesicles, measurements were made at four different vesicle concentrations. At each concentration, $C(\tau)$) was calculated for five τ values, each $C(\tau)$ being the mean of five to ten counts of scattered light intensity.

Water permeability

Stop-flow measurements were performed on a temperature controlled Bio-Logic SFM2. For each run, the data of scattered light intensity were simultaneously acquired on two channels: the first run duration being of 1 s with a acquisition rate of 1 kHz, the second being of 5 s (0.2 kHz acquisition rate) in order to have a good precision on the beginning and the overall evolution of the phenomenon. Unless otherwise specified, experiments were done at 26°C, the temperature of our previous in vitro microperfusion experiments [2].

0.1 ml of freshly prepared vesicles (0.4 mg protein/ml) were mixed with an equal amount of hyperosmotic solution in the stop-flow apparatus to reach an inwardly directed gradient of 50 mosM. Osmotic equilibration between the inside and outside of the vesicle was reached by water loss. Shrinkage of the sealed vesicles thus induced a change in the scattered light intensity. The proportion of unsealed BLMV has previously been measured by detergent activation of Na^+/K^+-ATPase activity and [^{3}H]ouabain binding [17] and showed that 50–65% of the BLMV obtained by centrifugation on a Percoll gradient were leaky. These vesicles contribute to the scattered intensity but not to the change in intensity as they are osmotically inactive. Their presence simply increases the noise. To maximize the signal-to-noise ratio, a low osmolality (27 mosM) was chosen for the buffer in which the vesicles were prepared in order to have larger volume changes at small gradients. This, moreover, slows down the time course of vesicle volume change.

Data of changes in 90° scattered light intensity ($\lambda = 350$ nm) as a function of time were averaged over at least three runs and stored on a AT Tandon Personal

Computer for subsequent analysis. A 150 W halogen lamp was used for maximal stability of the light source.

The time curves obtained when vesicles were mixed with isoosmotic buffer was substracted from all experimental curves before analysis.

Calculations of P_f

Optical signals were fitted to a single exponential function by the Pladé-Laplace method [29]:

$$S = S_\infty - \Delta S \exp(-kt) \quad (3)$$

ΔS, the amplitude of the change in scattered light intensity during the osmotic vesicle shrinkage is a function of the refractive index of the medium in which the vesicles are suspended [15], offset settings, concentration of vesicles and amplitude of the imposed osmotic gradient. k, on the other hand, depends exclusively on membrane permeability, vesicle geometry and osmolality of the hyperosmotic solution used to induce water outflow. The osmotic permeability (P_f) as demonstrated by Heeswijk and Van Os [8] can be expressed as:

$$P_f = \frac{kV_0\,(1 - b/V_0)}{\sigma V_w A C_m} \quad (4)$$

k being the rate constant of the exponential, V_0 the initial vesicular volume, A the membrane area, C_m the osmolality of the hyperosmotic medium, V_w the molar volume of water, σ the reflection coefficient of the solute present in the hyperosmotic solution and b the osmotically inactive volume. b/V_0, much smaller than one, was neglected.

For water permeability measurements, water was extracted by submitting the vesicles to a gradient of mannitol, a poorly permeant solute, the reflection coefficient of which is assumed to be one.

Determination of salt reflection coefficient (σ)

Vesicle shrinkage was induced by a gradient of the studied salt (NaCl or KCl) or by an identical gradient of mannitol taken as reference (σ(mannitol) = 1). Considering Eqn. 4, the solute reflection coefficient can then be expressed as:

$$\sigma(\text{solute}) = \frac{k(\text{solute})}{k(\text{mannitol})} \quad (5)$$

As mentioned above, k is not refractive index dependent. There is therefore no need for refractive index corrections by addition of pyrrovinyl pyrrolidone or other high molecular weight polymers to match salt and mannitol refractive indexes [15].

BBMV and BLMV are known to be permeable to NaCl and KCl but as the halflife of NaCl and KCl entrance are two orders of magnitude larger than the half-life of water outflow (unpublished results), the entrance of salt can be neglected in the studied time range of vesicle shrinkage.

Data are expressed as means ± S.E. and the paired Student's t-test was used for statistical analysis ($P < 0.01$).

Results

Characterization of vesicle preparations

Specific activities of marker enzymes are given in Table I). A 17-fold enrichement in Na^+/K^+-ATPase in the upper band on the Percoll gradient indicated a purified basolateral membrane fraction. The two other vesicle batches were found to be brush border (11-fold enrichment in maltase) and endoplasmic reticulum (6-fold enrichment in glucose-6-phosphatase) membranes. All three fractions were freed of mitochondrial membrane contamination.

When comparing the data in Table I with the enrichment values published by other investigators (using different isolation techniques), the separation of the three types of membrane with the present Percoll gradient method leads to a better enrichment in Na^+/K^+-ATPase for the BLMV [30] fractions and a similar degree of purification of the BBMV [7] and ERMV [21].

TABLE I

Specific activities and enrichments of marker enzymes

The specific activities are expressed in μmol per mg protein per h. BBMV/H, BLMV/H and ERMV/H are enrichments in enzyme activity as compared to the homogenate in brush-border, basolateral and endoplasmic reticulum vesicle preparations, respectively.

Enzyme	Homogenate	BBMV	BLMV	ERMV	BBMV/H	BLMV/H	ERMV/H
Glucose-6-phosphatase (endoplasmic reticulum)	2.91 ± 0.22 ($n = 6$)	8.06 ± 1.09 ($n = 6$)	5.98 ± 0.74 ($n = 6$)	15.78 ± 2.64 ($n = 4$)	2.69 ± 0.35	2.02 ± 0.28	5.77 ± 0.93
Maltase (BBM)	9.23 ± 1.47 ($n = 5$)	95.83 ± 15.49 ($n = 5$)	10.57 ± 1.73 ($n = 5$)	22.37 ± 5.17 ($n = 4$)	11.06 ± 1.00	0.09 ± 0.03	2.19 ± 0.36
Na^+/K^+-ATPase (BLM)	2.53 ± 0.25 ($n = 9$)	8.16 ± 0.76 ($n = 9$)	41.10 ± 3.18 ($n = 9$)	1.80 ± 0.33 ($n = 3$)	3.34 ± 0.34	16.58 ± 0.59	0.93 ± 0.27
Succinate dehydrogenase (mitochondria)	3.52 ± 0.33 ($n = 9$)	1.73 ± 0.24 ($n = 9$)	0.91 ± 0.13 ($n = 9$)	3.06 ± 0.80 ($n = 4$)	0.52 ± 0.06	0.26 ± 0.03	0.77 ± 0.19

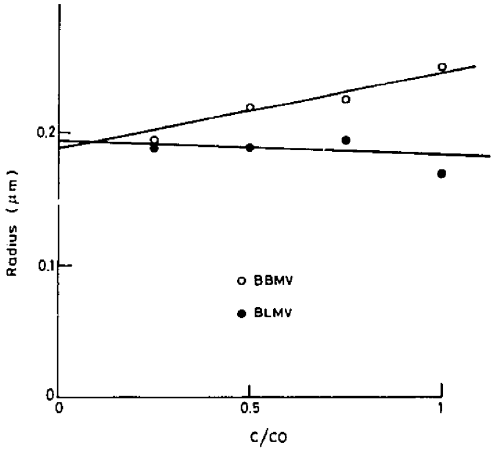

Fig. 1. Determination by quasi-elastic light scattering of the mean vesicle radius of a vesicle population. The light scattered by a population depends on the size of the vesicles and their mutual interactions. C/C_0 is a normalized concentration, C_0 being the concentration of the most concentrated batch. The extrapolation of the apparent radius to a zero vesicle concentration leads to an exact size determination. In this ideal situation no interactions between vesicles interfere with the measurements.

The advantage of this method is that the three types of membrane can be isolated from the same rabbit.

Vesicle sizes

QELS size measurements were performed on aliquots of freshly prepared brush-border, basolateral and endoplasmic reticulum membrane vesicles. Interactions between vesicles induce a perturbation of the scattered light and lead to an apparent hydrodynamic radius (R_{Happ}) value. Measurements were therefore performed at four different protein concentrations for each type of membrane vesicle and the correct value R_H was obtained from a linear extrapolation of R_{Happ} to a zero concentration. This corresponds to the ideal situation in which a single vesicle is suspended in the buffer. A typical experiment is shown Fig. 1. Paired values of BBMV and BLMV radii values are amazingly constant in any given experiment (Table II). The variations from one experiment to the other are small (170–210 nm radius). On the other hand, ERMV have repeatedly radii values that are significantly different from BBMV and BLMV radii. The mean radius was 193 ± 10 nm for BBMV ($n = 7$), 185 ± 3 nm for BLMV ($n = 7$) and 136 ± 10 nm for ERMV ($n = 4$).

Osmotic permeabilities

Fig. 2 shows the change in scattered light intensity when BBMV, BLMV and ERMV are mixed with a hyperosmotic medium to reach an inwardly directed gradient of 50 mosM of mannitol. All data fit to a single-exponential function.

TABLE II

Vesicle sizes

For each type of vesicle, the mean radius of the population was measured by quasi-elastic light scattering. The values are the extrapolation to a zero vesicle concentration.

Vesicle	Radius (nm)		
	BBMV	BLMV	ERMV
	188	185	
	170	172	
	201	197	
	210	188	140
	190	189	162
	234	192	128
	156	176	115
Mean	193 ± 10 ($n = 7$)	$185 + 3$ ($n = 7$)	136 ± 10 ($n = 4$)

Table III shows the mean half-life $t_{1/2}$ of exponential kinetics of water outflow (defined as $t_{1/2} = \ln 2/k$) and the corresponding P_f values of BBMV and BLMV over 10 experiments from 10 different rabbits (see also Fig. 3). The mean values that are given in Fig. 4 correspond exclusively to the four experiments were the three types of membrane were simultaneously isolated. According to the paired Student's t-test water permeability of BLMV is significantly higher than that of BBMV (35% difference). P_f is 123 ± 8 μm/s for BBMV ($n = 10$), 166 ± 10 μm/s for BLMV ($n = 10$) and 156 ± 9 μm/s for ERMV ($n = 4$).

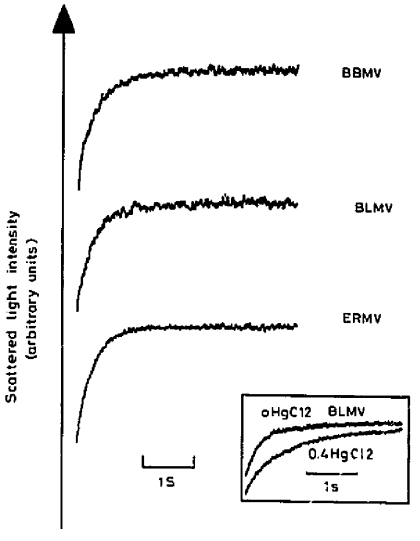

Fig. 2. Time courses of BBMV, BLMV and ERMV water shrinkage. Vesicles (0.4 mg/ml) in 20 mM mannitol, 2 mM Tris-Hepes (pH 7.4) at 26 °C were mixed in a stop-flow apparatus with a hyperosmotic solution in order to reach an inwardly directed gradient of 50 mM of mannitol. In the inset: BLMV in buffer solution were subjected to an inwardly directed gradient of 50 mM of mannitol (26 °C) in presence and absence of $HgCl_2$ (0.4 mM).

TABLE IIIa and IIIb

Kinetic parameters of water outflow and influence of $HgCl_2$

The mean half-life $t_{1/2}$ ($t_{1/2} = \ln 2/k$) of water outflow kinetics and the corresponding osmotic water permeability are given for each type of membrane. To measure the effect of $HgCl_2$, vesicles were incubated with 0.4 mM $HgCl_2$ and immediately subjected to an inwardly directed gradient of 50 mosM of mannitol in the stop-flow observation cell.

Parameter	BBMV	BLMV
$t_{1/2}$ (ms)	267 ± 17 (n = 10)	195 ± 10 (n = 10)
P_f (μm/s)		
Control	123 ± 8 (n = 10)	166 ± 10 (n = 10)
0.4 mM $HgCl_2$	54 ± 7 (n = 6)	65 ± 4 (n = 6)

	P_f (μm/s)		
	BBMV	BLMV	ERMV
Control	124 ± 8 (n = 4)	163 ± 12 (n = 4)	156 ± 9 (n = 4)
0.4 mM $HgCl_2$	53 ± 11 (n = 4)	65 ± 6 (n = 4)	61 ± 4 (n = 4)

Effect of $HgCl_2$

We have previously shown that water permeability of rat kidney brush-border membranes can be reversibly

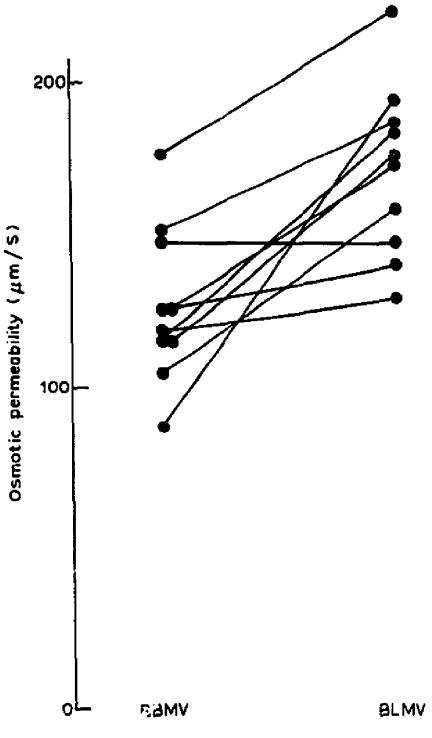

Fig. 3. Comparison of the osmotic water permeabilities of BBM and BLM vesicles. BBMV and BLMV isolated from the same rabbit kidney by the same technique are joined by a line.

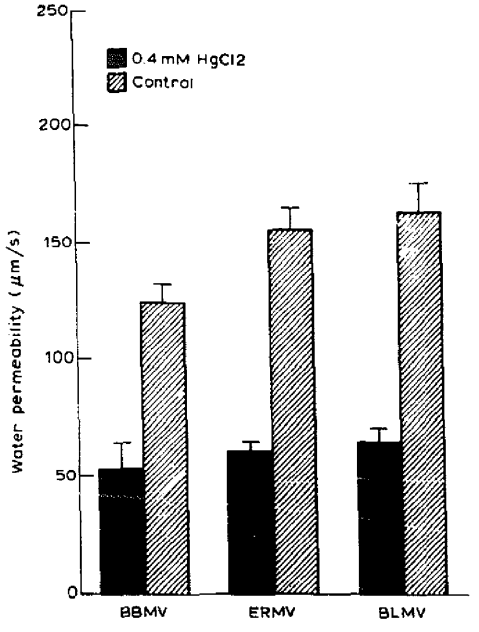

Fig. 4. Influence of 0.4 mM $HgCl_2$ on the osmotic permeability of BBMV, ERMV and BLMV. P_f were mean values obtained when considering four experiments where BBMV, BLMV and ERMV and ERMV were all three isolated from one rabbit.

inhibited by mercurial sulfhydryl reagents [7]. The effect of $HgCl_2$, which was clearly the most potent inhibitor, was therefore studied on BBMV, BLMV and ERMV. The inset in Fig. 2 illustrates the typical effect of 0.4 mM $HgCl_2$ on osmotic shrinking. In BBMV, ERMV and BLMV, P_f was decreased 57% ($P_f = 54 \pm 7$ μm/s, $n = 6$), 60% ($P_f = 65 \pm 4$ μm/s, $n = 6$) and 61% ($P_f = 61 \pm 4$ μm/s, $n = 4$), respectively (Fig. 4)

Reflection coefficients

σ(NaCl) and σ(KCl) were determined by comparing BBM, BLM and ERM vesicle shrinkage induced by an inwardly-directed 50 mosM gradient of NaCl or KCl to the volume change induced by an equiosmolar amount of mannitol. For the three considered solutes, time curves were exponential functions. With the constant

TABLE IV

Reflection coefficients of NaCl and KCl in BLMV, BBMV and ERMV

Reflection coefficients were obtained by comparisons of the kinetics of vesicle shrinkage induced by mannitol and the studied solute. None of the obtained coefficients was significantly different from 1.

	BBMV	BLMV	ERMV
σ(NaCl)	1.10 ± ±0.07 (n = 9)	1.05 ± 0.08 (n = 6)	0.97 ± 0.02 (n = 4)
σ(KCl)	0.97 ± 0.08 (n = 5)	1.04 ± 0.14 (n = 6)	0.98 ± 0.04 (n = 4)

rates so obtained, the reflection coefficients of the two salts were calculated from Eqn. 5. As reported in Table IV, both NaCl and KCl had reflection coefficients not significantly different from one with all the membranes studied.

Discussion

To get an estimate of the amplitude of transcellular water permeability of the rabbit proximal convoluted tubule, osmotic permeabilities of the brush-border and the basolateral membranes were required. The present study determined values of the water permeabilities of individual membranes finding 123 μm/s for BBMV and 166 μm/s for BLMV. Fig. 3 shows that in a given kidney the basolateral membrane is always slightly more permeable to water than the brush-border membrane, which seems to be the limiting barrier to water transport. The 35% difference between the two plasma membranes in the present study was smaller than that mentioned by Verkman and Ives [30] and Meyer and Verkman [32] who found the basolateral membrane to be at least twice as permeable to water as the luminal membrane ($P_f = 73$ μm/s for BBMV and 190–250 μm/s for BLMV at 23°C). The discrepancies between their and our results may be due to the fact that their brush-border and basolateral membranes were isolated by two different methods and from different rabbits. Also, their measurements of the BBMV and BLMV radii led to different values (0.3 μm for BBMV and 0.5–0.6 μm for BLMV) whereas the BBMV and BLMV in the present study were of similar size.

A possible explanation for the recorded difference in P_f values of BBMV and BLMV would be that brush-border and basolateral membrane preparations may be contaminated by membranes derived from intracellular organelles which could have influenced the permeability measurements. Thus if intracellular membranes were less permeable to water than plasma membranes, the mean permeability recorded from a contaminated preparation would be an underestimation of the true permeability. As intracellular membranes have rarely been isolated from proximal rabbit tubules however, the actual effect of contamination on permeability data has never been quantified. We therefore measured the water permeability of a membrane fraction enriched in endoplasmic reticulum. Unexpectedly we again found an extremely high water permeability mediated by water channels showing that: (1) high P_f values are not specific to renal plasma membranes but common to intracellular membranes, (2) ERMV, BBMV and BLMV water permeability properties are so similar that slight cross contamination of plasma membrane preparations by endoplasmic reticulum membranes would not affect P_f measurements.

How do these membrane osmotic permeabilities compare to transepithelial permeabilities? Assuming an exclusively transcellular water flow in which the only resistances would be the brush-border and the basolateral membranes acting in series (we will neglect the resistance of the cytoplasm) a theoretical transcellular permeability ($P_{f_{theo}}$) can be calculated. $P_{f_{theo}}$ is related to the experimentally measured apical (P_{f_a}) and basolateral (P_{f_b}) water permeabilities by the relation:

$$1/P_{f_{theo}} = 1/P_{f_a} + 1/P_{f_b} \quad (6)$$

No surface area corrections are necessary, as Welling and Welling [33] showed that the brush-border and the basolateral membranes have the same surface areas. The present data (123 μm/s for BBMV and 166 μm/s for BLMV) led to a $P_{f_{theo}}$ of 71 μm/s.

In order to be able to compare $P_{f_{theo}}$ with transepithelial water permeability values of the proximal convoluted tubule obtained using in vitro microperfusion techniques, we will briefly review how the different values summarized in Table V were obtained. Kokko et al. [34] were the first to measure water movement in perfused rabbit proximal tubules by creating an osmotic gradient between lumen and bath. Later, Schafer et al.

TABLE V

Values of osmotic water permeabilities in rabbit proximal tubule

All data are expressed in μm/s. The P_f values obtained by microperfusion, expressed in column 3 per unit of apparent apical surface area, were converted to μm/s per unit of membrane area considering the surface area of the brush border membrane as that measured by Welling and Welling [33] which takes microvillosities into account (column 4).

Investigators	Temp. (°C)	Original transepithelial P_f (μm/s)	Transepithelial P_f per unit of membrane area (μm/s)
Kokko [34]	37	402– 686 [a]	9–15
Corman and Di Stefano [2,13]	26	728–1600	21–42
Barfuss et al. [38]	38	880–1200	24–33
Schafer et al. [35]	25	1290–2420 [a]	30–57
Berry and Verkman [37]	26	3300 [b]	71

[a] The reflection coefficient of NaCl, when used in *Pf* calculations, was taken equal 1.

[b] Mean of all values obtained at 26°C with an osmotic gradient of 20 mosM.

[35] noticed that measured P_f values were perfusion rate dependent indicating a nonlinear profile of osmotic equilibration along the perfused tubule. Taking their values of the highest experimentally tested perfusion rate or the extrapolation to an infinitely high perfusion rate which would correspond to a uniform gradient between lumen and bath along the tubule, they obtained a P_f between 1290 and 2420 μm/s (with $\sigma(NaCl) = 1$) [1]. A more mathematical approach was proposed by Dubois et al. [36] who established an equation approximating the nonlinear profile of the osmotic pressure along the tubule. Berry and Verkman [37] and Corman and Di Stefano [13] adopted this solution to overcome the underestimation of P_f at a low perfusion rate and also considered new parameters.

Measured osmotic permeabilities were shown by Berry and Verkman [37] also to be dependent on the gradient between lumen and bath during experiments: the smaller the gradient the higher the apparent permeability. Their reported mean P_f value was 3300 μm/s at a gradient of 20 mosM. The phenomenon of unstirred layers (USL) described by Dainty [38] which would result in underestimated water permeability values was investigated by Corman and Di Stefano [13] on the basis of electrophysiological principles. The argument was the following: the sweeping away effect due to water movement in the USL should lead to a dilution potential proportionate to the P_{Na}/P_{Cl} of the tubule. Then from the values of P_{Na}/P_{Cl} and the streaming potential induced by transepithelial osmosis across the tubular wall, the possible dilutions of the osmotic probe and of the bath solution in the presumed USL was calculated. For the extreme case corresponding to a USL all along the basolateral membrane, the measured P_f was found to underestimate the true value just by half. From the data of Corman [2], the P_f value would in that case be between 738 and 1600 μm/s.

In addition to the classical approach using steady-state flow measurements, an interesting method was adopted by Barfuss and Schafer. [39]: rabbit proximal convoluted tubules were perfused under oil so that droplets of absorbate could be collected. From measurements of the osmolality of the absorbate and volume reabsorption, P_f was determined and found to be in the range of 880–1200 μm/s.

The above values corresponded to water fluxes brought back to the inner surface of a perfused tubule considered as a cylinder. The inner diameter, used in P_f calculations varied according to the investigators from 20 [35,37] to 25 μm [39]. This parameter is probably dependent on the hydrostatic pressure imposed during perfusion. For comparing one study with another, it would seem more accurate to consider the exchange surface between lumen and bath, that is, the total apical cell surface, taking into account the folding factor due to the brush border. This surface is a morphological parameter which can be considered to be constant for rabbits and therefore independent of experimental conditions. The apical cell membrane surface, measured by Welling and Welling [33], which has the same area as the basolateral membrane is $2.9 \cdot 10^6$ μm^2/mm of tubule in the rabbit proximal convoluted tubule. Table V summarizes the P_f values obtained by recalculating the permeabilities using the absolute surface area of the brush border instead of the radius of the luminal cylinder (the area of the tight junctions was neglected). With this calculation, P_f values that had been between 1000 and 3000 μm/s fell into the range of 20 to 70 μm/s. The theoretical transcellular water permeability $P_{f_{theo}}$ calculated from the present experimental data is in the upper part of this 20–70 μm/s range measured on perfused tubules and supports the theory of a predominantly transcellular route for water transport in proximal convoluted tubule from rabbit kidney.

The second question we were interested in was: what mechanisms are responsible for the large water absorption along the proximal tubule? To point out the presence of water channels, several investigators [6–8,15,30] tested inhibition of water permeability in rat kidney brush border and basolateral membranes by mercurial reagents, as Solomon et al. [40] and Macey et al. [41] had succesfully done on the erythrocyte membrane. They found that water permeability was inhibited by about 60 to 80% by $HgCl_2$ or pCMBS. However, since both lipid bilayers and aqueous pores can account for the selectivity of a membrane to water, we checked whether mercurial compounds were specific to water channels or if they could inhibit the water permeability of artificial liposomes. Liposomes were obtained with the reverse phase evaporation technique using 50% of egg phosphatidylcholine and 50% cholesterol. They had a water permeability of 10 μm/s which remained unchanged when exposed to $HgCl_2$ (unpublished results). This water permeability inhibition by $HgCl_2$ can reasonably be used as a test to indicate the presence of proteic water channels. We applied this test to BBMV, BLMV and ERMV from rabbit kidney and confirmed previously published experiments by finding evidence for water channels in both luminal and basolateral membranes and, surprisingly, also in endoplasmic reticulum membranes.

The residual water permeabilities of BBMV and BLMV after inhibition by $HgCl_2$ were not significantly different (Fig. 4) and close to 60 μm/s. So the inhibitable component of the water permeability was 69 μm/s in BBMV and 101 μm/s in BLMV suggesting that a larger number of proteic channels are involved in water transport through the basolateral membrane than through the luminal membrane. The difference in water permeability that still remains, after inhibition by $HgCl_2$, between renal plasma membranes and liposomes containing a similar amount of cholesterol [42],

might be due to a difference in lipid composition or to the presence of water channels not inhibitable by mercurials in these cell membranes.

Our third interest in this work was to evaluate the interactions, if they exist, between water and ionic pathways. It has been previously shown that the osmotic water flux through the perfused proximal kidney tubule did not drag a significant amount of solute [2,14]. To explain this, at least one of the plasma membranes should have a solute reflection coefficient of one. In our study NaCl and KCl reflection coefficients were measured in BBMV, BLMV and ERMV and all σ values were not significantly different from one. This confirms what we have previously found in rat BBMV [15] and indicates that not only luminal but also basolateral and intracellular membranes have water channels that are not shared by salts.

In conclusion, the present BBMV and BLMV permeability data are compatible with a predominantly transcellular route for water movement. The luminal membrane seems to be the limiting barrier to transepithelial osmosis although the permeability values of the plasma membranes are not very different from one another. This high capacity for water transport is not specific to the plasma membranes since intracellular membranes from kidney tubular cells also have this characteristic. There is also evidence for the existence of proteic water specific pathways in all the membranes studied. These channels are probably not involved in salt transport as membrane reflection coefficients were close to one.

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