NaCl reflection coefficients in proximal tubule apical and basolateral membrane vesicles

Measurement by induced osmosis and solvent drag

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ABSTRACT Two independent methods, induced osmosis and solvent drag, were used to determine the reflection coefficients for NaCl (σ_{NaCl}) in brush border and basolateral membrane vesicles isolated from rabbit proximal tubule. In the induced osmosis method, vesicles loaded with sucrose were subjected to varying inward NaCl gradients in a stopped-flow apparatus. σ_{NaCl} was determined from the osmolality of the NaCl solution required to cause no initial osmotic water flux as measured by light scattering (null point). By this method σ_{NaCl} was >0.92 for both apical and basolateral membranes with best estimates of 1.0. σ_{NaCl} was determined by the solvent drag method using the Cl-sensitive fluorescent indicator, 6-methoxy-*N*-[3-sulfopropyl]quinolinium (SPQ), to detect the drag of Cl into vesicles by inward osmotic water movement caused by an outward osmotic gradient. σ_{NaCl} was determined by comparing experimental data with theoretical curves generated using the coupled flux equations of Kedem and Katchalsky. By this method we found that σ_{NaCl} was >0.96 for apical and >0.98 for basolateral membrane vesicles, with best estimates of 1.0 for both membranes. These results demonstrate that σ_{NaCl} for proximal tubule apical and basolateral membranes are near unity. Taken together with previous results, these data suggest that proximal tubule water channels are long narrow pores that exclude NaCl.

INTRODUCTION

The reflection coefficient is a nonequilibrium thermodynamic parameter which quantitates the degree to which solute and solvent interact as they traverse a membrane. The NaCl reflection coefficients (σ_{NaCl}) in plasma membranes of the renal proximal tubule cell are of particular physiological significance because their values have important implications for: (*a*) the mechanism of isosmotic reabsorption of volume and salt (Andreoli and Schafer, 1978), (*b*) the physical properties of the proximal tubule water channel (Finkelstein, 1987), and (*c*) possible mechanisms of cell volume regulation (Welling and Welling, 1988). There is strong evidence for the presence of water channels in both the apical and basolateral membranes in the proximal tubule (Meyer and Verkman, 1987; Verkman, Dix and Seifter, 1985; Pratz, Ripoche and Corman, 1985). However, it is not known whether these channels can accomodate and transport the major solutes present in tubular fluid. There have been numerous conflicting reports on the value of transepithelial σ_{NaCl} in the proximal tubule ranging from 0.36 to 1.0 (Rector et al, 1966; Corman and Distephano, 1983; Gonzalez et al., 1982). Recently, two reports of σ_{NaCl} determination in individual apical and basolateral membranes have appeared. Welling et al. (1987) reported a value for σ_{NaCl} of 0.5 in the basolateral membrane using a video technique to follow cell volume changes in the lumen collapsed proximal tubule. Pratz et al. (1986) reported a value of 0.5 for rat apical membrane vesicles by comparing the initial rate of volume change induced by NaCl with that induced by sucrose. The rapidity of osmotic water movement and the confounding effects of solution refractive index made equivocal the interpretation of the data in these studies.

We have developed two independent methods for the determination of σ_{NaCl} in brush border (BBMV) and basolateral (BLMV) membrane vesicles isolated from rabbit renal cortex. In the first method (induced osmosis), the effective osmotic strength (i.e., the product of reflection coefficient and cryoscopic osmolality) of a NaCl solution relative to a sucrose solution, (which has unity reflection coefficient) was determined. Vesicles containing sucrose were mixed in a stopped flow apparatus with NaCl solutions of varying osmolality. The cryoscopic osmolality of the NaCl solution required to cause no initial volume flux (null point) gives directly σ_{NaCl}. The second method makes use of the Cl-sensitive fluorescent indicator, 6-methoxy-*N*-[3-sulfopropyl]quinolinium (SPQ), to compare Cl influx in the presence and absence of osmotic water influx. Both the induced osmosis and solvent drag methods were sufficiently rapid and sensitive to measure the transients in volume and solute flux

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necessary for the accurate determination of σ_{NaCl}. The measurement of solvent drag by SPQ fluorescence represents a new technique with significantly greater sensitivity for determination of σ_{NaCl} than previously has been possible. Using the complementary induced osmosis and solvent drag approaches, we find that σ_{NaCl} is near unity in both apical and basolateral membranes from proximal tubule.

METHODS

Experimental design

Experimental protocols were designed on the basis of theoretical analyses of the optimal conditions for each of the two methods employed. The induced osmosis method relies on light scattering to follow instantaneous vesicle volume changes after vesicles, loaded with sucrose, have been mixed with varying concentrations of NaCl solution. Sucrose has unity reflection coefficient and thus serves as a reference solute against which the effective osmotic strength of the NaCl solution can be measured. Because the overall volume change is determined by the ratio of intra- to extravesicular osmolalities while the rate of volume change is determined by the absolute magnitude of the osmotic gradient, conditions of low osmolality maximized the sensitivity of this method. Near the null point the volume change is small and, at high osmolalities, very fast, causing reduction of the signal-to-noise ratio. For a given ratio of osm_{in}/osm_{out}, the total volume movement at low osmolalities is the same as at high osmolalities but occurs more slowly and can be discerned easily from background noise. The noise level is reduced further at low osmolalities by the lower refractive index. The induced osmosis method experiments were therefore conducted with 30 mOsm buffered sucrose solution in the intravesicular space.

In the solvent drag experiments Cl influx into vesicles was monitored by the Cl-sensitive fluorescent indicator, SPQ entrapped within the vesicles. Maximal sensitivity of this technique required a large inwardly directed NaCl gradient while maintaining a sufficiently large outwardly directed osmotic gradient (inside > outside osmolality) to drive water influx. Vesicles were loaded with 500 mOsm buffered sucrose solution and were mixed in the stopped flow apparatus with solutions containing equal NaCl concentrations at high and low osmolalities (set by sucrose). Chloride entry by diffusion and by solvent drag could thus be resolved. To maintain high oppositely directed NaCl and sucrose gradients, we used a 5:1 (NaCl test solution:vesicle solution) mixing ratio. The sensitivity of the solvent drag technique was intrinsically very high because 8 mM intravesicular Cl causes 50% quenching of SPQ fluorescence (Chen et al., 1988); 1 mM Cl causes 10% quenching of SPQ, which is easily measured.

Vesicle preparation

BBMV were isolated from the renal cortex of 2–2.5 kg New Zealand white rabbits using Mg aggregation and differential centrifugation as described previously (Verkman et al., 1985). BBMV were enriched >15-fold in maltase activity (apical marker) and <0.3-fold in ouabain-inhibitable ATPase activity. Basolateral membrane vesicles were obtained from renal cortex using the sucrose density gradient method (Meyer and Verkman, 1987). BLMV were enriched 15-fold in ouabain-inhibitable ATPase activity and <0.3-fold in maltase activity. Vesicles were used immediately after preparation or frozen at −70°C for later use.

Experimental protocols

Experiments were performed using a SF51 stopped-flow apparatus (Hi-Tech Wiltshire, England) with <2 ms dead time. Data acquisition and analysis were performed on a MINC/23 computer (Digital Equipment Corp., Maynard, MA). Data acquisition rate was 4 ms/point. Solution osmolalities were measured using a 3W2 freezing point depression osmometer (Advanced Instruments Inc., Needham Heights, MA) or a 5100 vapor pressure osmometer (Wescor Inc., Logan, UT) for osmolalities down to 50 mOsm. Measured osmolalities were compared with osmolalities computed using the van't Hoff coefficients for NaCl and sucrose. Measured osmolalities were within 5% of computed osmolalities for all solutions. Below 50 mOsm the osmometer loses accuracy. Computed osmolalities were used for the data analysis.

A. Induced osmosis

Vesicles were preincubated in >16 vol of 15 mM sucrose, 2.5 mM Hepes/Tris, pH 7.0 for 24 h at 4°C. Before use, vesicles were centrifuged at 300,000 *g* for 10 min, homogenized by 10 passages through a 23 gauge needle and three passages through a 26 gauge needle, and resuspended at a concentration of ~0.4 mg protein/ml. Just before mixing in the stopped-flow apparatus, vesicles were pre-shrunk to 50% of their initial volume in 30 mM sucrose, 5 mM Hepes/tris pH 7, to eliminate membrane inelasticity or cytoskeletal restriction as possible barriers to swelling. To verify that vesicles did not swell between pre-shrinking and use, separate experiments were performed in which vesicles (before pre-shrinking) were mixed in the stopped-flow apparatus with the preshrink solution; volume was followed by light scattering for 180 s. Vesicles swelled <3% back toward their initial volume. Induced osmosis experiments were begun by the rapid mixing in the stopped-flow apparatus of 0.075 ml of solution containing the preshrunk vesicles with an equal volume of solutions with varying concentrations of NaCl. The time course of vesicle volume change was followed by light scattering at 510 nm. Under these conditions, the intensity of scattered light has been shown previously to increase linearly with decreasing vesicle volume.

B. Solvent drag

SPQ was synthesized by reaction of propane sultone and 6-methoxy quinoline (Aldrich Chemical Co., Milwaukee, WI), as described previously (Krapf et al., 1988*a*). Vesicles were loaded with SPQ by incubation in 240 mM sucrose, 10 mM SPQ, 5 mM Hepes/Tris, pH 7.0 for 48 h at 4°C. Extravesicular SPQ was removed by three washes in 250 mM sucrose, 5 mM Hepes/Tris, pH 7. Washed vesicles were preshrunk in 500 mM sucrose 5 mM Hepes/Tris and mixed in the stopped-flow apparatus in a 5:1 ratio with either a hyposmotic or isosmotic NaCl solution (as described above). Fluorescence was excited by a tungsten-halogen lamp through a 360 ± 20 nm six-cavity interference filter (Omega Optical Inc., Brattleboro VT) and a KG3 infrared blocking filter (Schott Glass Technologies Inc., Duryea, PA); emitted light was filtered by two 410 nm cut-on filters (Schott Glass Technologies Inc.).

The fluorescence signal from these experiments was normalized to the total intravesicular quenchable signal using KSCN to completely quench the intravesicular SPQ. Vesicles are permeable to SCN (equilibration time ~10 s); 125 mM SCN caused >99% quenching of SPQ (Illsley and Verkman, 1987). Vesicles were mixed with KSCN in the stopped-flow apparatus and fluorescence time course was followed over 45 s (at which time a stable signal had been reached). The amplitude of the SCN quenching curve was used to determine F_o, the total quenchable fluorescence signal.

Computer simulations

Quantitative determination of σ_{NaCl} was made by comparing the experimental curves for vesicle volume and Cl entry with theoretical curves generated by solution of the coupled nonequilibrium thermodynamic flux equations of Kedem and Katchalsky (1958) (in the absence of hydrostatic pressure):

$$J_v = -P_f(\Delta\pi_i + \sigma_{NaCl}\,\Delta\pi_s) \quad (1a)$$

$$J_s = (1 - \sigma_{NaCl})C_s J_v + P_s \Delta C_s, \quad (1b)$$

where J_v = volume flux (nl/s · cm^2), P_f = osmotic water permeability (cm/s), $\Delta\pi_i$ = osmotic gradient for impermeant solute (mOsm), and $\Delta\pi_s$ = osmotic gradient for permeant solute (mOsm), J_s = solute flux (mol/s · cm^2), P_s = solute permeability (cm/s), C_s = solute concentration taken to be the external Cl concentration (millimolar). These equations were solved by the forward Euler's method using compiled Basic on an IBM AT with an 80287 math coprocessor. For comparison with experiments, intravesicular [Cl] was converted to units of SPQ fluorescence by use of the Stern-Volmer relation for Cl:

$$F_o/F = 1 + K[Cl], \quad (2)$$

where F = SPQ fluorescence, F_o = total quenchable fluorescence and K is the Stern-Volmer constant for SPQ (118 M^{-1}; Illsley and Verkman, 1987). Values of P_s and P_f used in the simulations were obtained by comparison of the time constants for single exponential fits to the experimental data with time constants for single exponential fits to theoretical curves as previously described (Chen et al., 1988*b*). Conditions for P_s determination were as shown in Figs. 3 and 4: intravesicular osmolality was 500 mOsm, extravesicular osmolality was 280, and [NaCl] was 104 mM. For P_f determination, intravesicular osmolality was also 500 mOsm and extravesicular osmolality was 280; no NaCl was present. Surface-to-volume ratios were 2×10^5 cm^{-1} (BBMV) and 1.2×10^5 cm^{-1} (BLMV) (Meyer and Verkman, 1987) and modified for the initial conditions of these experiments (where vesicles are pre-shrunk to half of their usual volume).

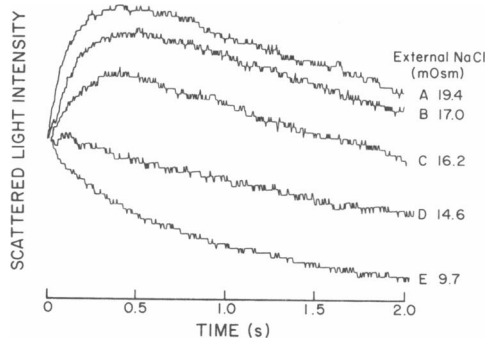

FIGURE 1 Determination of BBMV σ_{NaCl} by null point method. BBMV were subjected to a 15 mOsm outwardly directed sucrose gradient and varying inward NaCl gradients in a stopped flow apparatus. The time course of scattered light intensity, which increases linearly with decreasing vesicle volume, was followed. Conditions after 1:1 mixing in the stopped-flow apparatus were: intravesicular- 30 mM sucrose, 5 mM Hepes/Tris; extravesicular- 15 mM sucrose 5 mM Hepes/Tris, plus varying osmolalities of NaCl as shown. These results are typical of two sets of experiments performed on separate preparations. Each curve is the average of six separate experiments.

RESULTS

A. Induced osmosis

The null point was determined by the osmolality of a NaCl solution which caused no initial volume flux when mixed with vesicles containing an impermeant solute with unity reflection coefficient (sucrose). σ_{NaCl} was determined from the osmolality of an NaCl solution at the null point by setting $J_v = 0$ in Eq. 1a to obtain:

$$\sigma_{NaCl} = -\pi_i/\pi_s, \quad (3)$$

where π_i is the transmembrane osmotic gradient for sucrose, and π_s is the osmotic gradient for NaCl. A null point experiment for BBMV is shown in Fig. 1. The NaCl solution osmolalities shown represent calculated osmolalities after one-to-one mixing in the stopped flow apparatus. The van't Hoff coefficient for NaCl at these concentrations is 1.94 and does not change significantly during the course of the experiment.[1]

In Fig. 1, curves *A–C*, the vesicles shrink initially, indicating that the extravesicular effective osmolality (product of σ_{NaCl} and cryoscopic osmolality) is greater than intravesicular osmolality. In curve *D* there is a slow progressive swelling of vesicles due to NaCl entry and consequent volume influx, but there is no initial shrinkage (as seen in curves *A–C*) or rapid swelling phase (as seen in curve *E*). In curve *E*, the vesicles have two phases of swelling, a rapid phase due to the greater intravesicular osmolality (resulting in rapid osmotic water influx) and a slow phase due to NaCl entry. Because the vesicles shrink in curve *C*, the null point must occur at an external osmolality <16.2. Therefore, a lower limit for σ_{NaCl} of 15/16.2 (=0.92) was determined from Eq. 3 using the sucrose and NaCl osmotic gradients from curve *C*. Each curve shown is the average of six separate determinations.

A best estimate for σ_{NaCl} was obtained by comparison of the experimental curves with theoretical curves generated by iterative solution of the nonequilibrium thermodynamic flux equations as described in Methods. Fig. 2 shows the results of simulations with σ_{NaCl} varying from

[1]The van't Hoff coefficient for NaCl varies slightly with vesicle volume because the NaCl concentration changes. If intravesicular [NaCl] changes by as much as 5 mM (see Figs. 3 and 4), the van't Hoff coefficient will have changed by <0.012. This will change intravesicular osmolality by <1% from that predicted by using a constant van't Hoff coefficient. The extravesicular NaCl concentration does not change. The van't Hoff coefficient for sucrose is unaffected by concentration over the range encountered in these experiments.

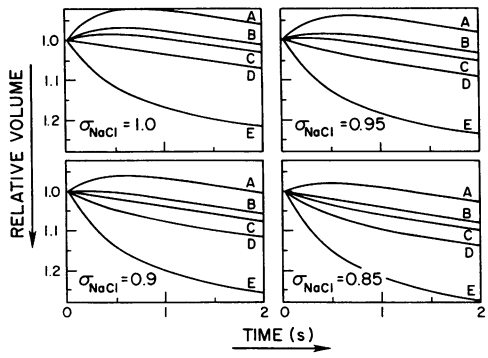

FIGURE 2 Computer simulations of the null point experiment for differing values of σ_{NaCl}. The mixing conditions and concentrations are as in Fig. 1. The nonequilibrium thermodynamic Eqs. 1, a and b were solved as described in methods. Values for P_f and P_s were 0.012 cm/s and 9×10^{-7} cm/s, respectively. The simulations shown are plotted with relative volume decreasing along the +y axis to facilitate comparison with the scattering data in Fig. 1.

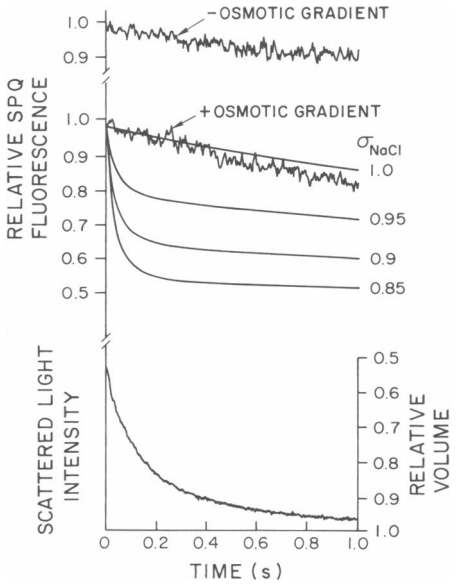

FIGURE 3 Determination of BLMV σ_{NaCl} by the solvent drag method. Cl influx in the presence and absence of osmotic water influx was measured by SPQ fluorescence quenching. In the top curve there is no osmotic gradient. Conditions after mixing in the stopped-flow are: intravesicular osmolality = 500 mOsm, [NaCl] = 0; extravesicular osmolality = 500 mOsm, [NaCl] = 104 mM. In the second experimental curve there is an outwardly directed osmotic gradient of 220 mOsm: intravesicular osmolality = 500 mOsm, [NaCl] = 0; extravesicular osmolality = 280 mOsm, [NaCl] = 104 mM. Curves were offset for clarity. Smooth curves represent theoretical time course of intravesicular [NaCl] generated for varying values of σ_{NaCl} as shown. Values of P_s and P_f used in the simulations were 1.3×10^{-7} cm/s and 0.015 cm/s, respectively. S/V_o was 6×10^4. NaCl concentration and osmotic gradient were as for the second experimental curve (+ osmotic gradient). The bottom tracing represents relative vesicle volume as determined by light scattering over the same time interval that SPQ fluorescence is being followed. These results are typical of three sets of experiments performed on separate preparations. Each curve is the average of 15 separate experiments.

1.0 to 0.85 and with initial conditions identical to the actual experiments in Fig. 1. The simulated curve shapes most closely match the experimental curves when σ_{NaCl} = 1.0. The curves obtained with σ_{NaCl} = 0.95 are also consistent with experimental data. However, the curves obtained with σ_{NaCl} = 0.90 are not (compare the initial direction of volume change in curve *c* with Fig. 1, curve *c*). This indicates that σ_{NaCl} is >0.90 with a best estimate of 1.0.

The results for BLMV were similar to those shown for BBMV in Fig. 1. The null point occurred between [NaCl] = 16.2 mOsm and [NaCl] = 15 mOsm giving a lower limit of 0.92 for σ_{NaCl}. These results place σ_{NaCl} for both the brush border and basolateral membranes at >0.92, with a best estimate of 1.0.

B. Solvent drag

As seen in Eq. 1b, if the reflection coefficient is <1, then solvent will drag solute as it traverses the membrane in response to an osmotic gradient. Fig. 3 shows the results of experiments and simulations for BLMV. Experimental curves show vesicle [Cl] as a function of time in the presence (+ osmotic gradient) and absence (− osmotic gradient) of an outwardly directed osmotic gradient. In the presence of the osmotic gradient, the vesicles swell rapidly to about twice their initial volume (which brings them back close to their volume before pre-shrinking). Fig. 3, (*bottom*) shows vesicle volume change as measured by light scattering at 510 nm over the same time interval as the SPQ measurements. It can be seen that water movement is >90% completed over 400 ms. If solvent drag were to occur, then the early water movement in the presence of an osmotic gradient would cause a rapid influx of NaCl followed by a slower diffusive component. The change in SPQ fluorescence over one second was the same in the presence or absence of an osmotic gradient indicating an absence of measurable solvent drag. Each curve is the average of 15 experiments. The results shown are typical of three sets of experiments. In each set of experiments 8 to 15 separate determinations were performed in generating each curve.

Quantitative determination of σ_{NaCl} was made by comparison of the experimental data with the theoretical curves shown in Fig. 3 (*smooth curves*). These curves were obtained by solution of Eqs. 1a, b, and 2 using parameters given in the figure legend. The nonlinear quenching characteristics of SPQ cause the simulated curves to have a steep initial slope when $\sigma_{NaCl} < 1$. In simulations not shown, σ_{NaCl} was varied from 1.0 to 0.95 by increments of 0.01. At a value for σ_{NaCl} of 0.98, the

theoretical curve was below the experimental curve and entirely outside of its noise envelope. This provided a lower limit for σ_{NaCl} of 0.98. Comparison of the initial slopes of the experimental and theoretical curves gave a best estimate for σ_{NaCl} of 1.0.

Fig. 4 shows the same experiment performed using BBMV. The findings are very similar to the BLMV results: osmotic water flux does not significantly augment early Cl movement. Comparison of initial slopes gave a best estimate for σ_{NaCl} of 1.0. Because each curve is the average of eight experiments, the noise envelope was slightly larger than in the basolateral membrane (where each curve was the average of 15 experiments). Using the same procedure as described for the basolateral membrane, the lower limit for σ_{NaCl} was 0.96.

Control studies were performed to show that there was no interference with the SPQ signal by scattered light or SPQ self-quenching. With excitation at 360 and emission at >410, neither swelling nor shrinking of BBMV or BLMV in the absence of Cl caused a measureable change in fluorescence signal. The effect of changing SPQ concentration (which would occur during rapid swelling) on the Stern-Volmer relation was tested in the fluorimeter by determining the Cl quenching curve at varying concentrations of SPQ. There was no effect of SPQ concentration between 0.5 and 20 mM, and thus there should be no effect of vesicle swelling on the quenching characteristics of the SPQ. Finally, light scattering experiments performed in the presence and absence of SPQ showed that SPQ did not itself alter BBMV or BLMV P_f and P_s.

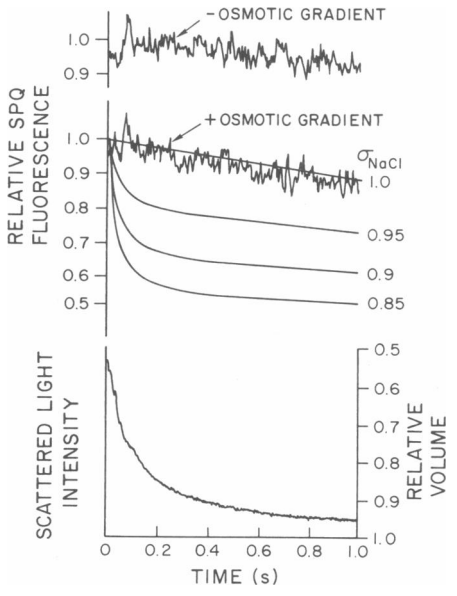

FIGURE 4 Determination of BBMV σ_{NaCl} by the solvent drag method. Conditions are as in Fig. 2. Values of P_f and P_s were 0.01 cm/s and 1×10^{-7} cm/s, respectively. S/V_0 was 1×10^5. These results are typical of four sets of experiments performed on three separate preparations. Each curve is the average of eight separate experiments.

It has been shown previously that both BBMV and BLMV have water channels which are functionally similar to those found in intact cell membranes; high P_f, low activation energy, and inhibition by mercurials (Meyer and Verkman, 1987). We confirmed the presence of water channels in our vesicle preparation by performing light scattering experiments at 510 nm (well above the excitation wavelength for SPQ). With 280 mOsm externally and 500 mOsm internally, BBMV and BLMV swell with exponential time constants at 100 and 120 ms, respectively, giving P_f values of 0.01 and 0.015 cm/s for pre-shrunk BBMV and BLMV, respectively (as shown in Figs. 3 and 4, *bottom panels*). These values are comparable with those reported previously. BBMV water transport was inhibited 50–60% by 0.5 mM HgCl2, which is comparable with inhibition reported previously for these vesicles (Meyer and Verkman, 1987). In BLMV, inhibition was 30–50% which is slightly less than previous reports (50–60%). However, activation energy for water transport in the BLMV was 3.8 kcal/mol which is very close to values reported previously and supports the presence of water channels (Meyer and Verkman, 1987).

DISCUSSION

We have determined the NaCl reflection coefficients in brush border and basolateral membrane vesicles from rabbit proximal tubule by the induced osmosis method, and by a novel fluorescence method for measuring solvent drag. Previously, widely varying values of transepithelial and isolated apical and basolateral membrane NaCl reflection coefficients have been found, ranging from 0.36 to 1.0 (Rector et al., 1966; Welling et al., 1987; Pratz et al., 1986; Gonzalez et al., 1982). Moreover, transepithelial solvent drag has been demonstrated under steady state conditions in some intact tubule studies (Andreoli et al., 1979), but not in others (Corman and Di Stefano, 1983; Jacobson et al., 1982). Solvent drag has not been assayed previously in any cell or vesicle membrane because of the lack of a suitably rapid system to measure early transients in solute flux. The results of these studies demonstrate that solvent drag of NaCl does not occur in the apical or basolateral membranes and establish from two independent approaches that the NaCl reflection coefficients are >0.92 with a best estimate of 1.0.

Light scattering, which was used in our null point determination, is subject to refractive index artifacts which introduce nonvolume dependent effects on the signal amplitude (Mlekoday et al., 1983). This presents a potentially large source of error in experiments that rely on the initial rate of volume flux to determine σ_{NaCl}. Under

these conditions, a small difference in refractive index between the experimental and control solutions can affect greatly the slope of the initial intensity versus time curve and can therefore cause large errors in the value of σ_{NaCl}. The null point method minimizes the effect of refractive index artifacts because the direction and not the rate of the initial signal is used to determine σ_{NaCl}. Refractive index effects were reduced further in our experiments by the low solute concentrations used. The use of sucrose as a reference solute with unity reflection coefficient for both the null point and solvent drag experiments is based on our and previous investigators' observations of the near impermeability of cell membranes for it.

The NaCl reflection coefficient for rat BBMV was recently reported by Pratz et al. (1986). They used light transmission (subject to the same refractive index artifacts as scattering) to measure rates of vesicle volume change in response to an osmotic gradient. By comparing the initial rate of volume flux in response to a NaCl gradient with that induced by a sucrose gradient they obtained a value for σ_{NaCl} of 0.5 in contrast to our value of 1.0. This difference could be due to species difference or to a difference in the techniques used. The most likely source of error however was from refractive index artifacts, which are minimal using the null point method.

SPQ has been used previously to measure both steady state and transient Cl concentrations in both cells (Krapf et al., 1988*b*) and vesicles (Chen et al., 1988*a*; Illsley and Verkman, 1987). Results have been validated by comparison with radioactive tracers (Chen et al., 1988*a*) and with results previously reported in the literature. SPQ reports [Cl] accurately and has no effect on Cl transport characteristics in any of the systems studied. In our experiments, SPQ had no effect on P_f or P_s nor did it alter the ability of $HgCl_2$ to inhibit the water channel. SPQ provides a rapid (response time <1 ms) and sensitive means of detecting small amounts of Cl influx. With SPQ, a change in [Cl] of 1 mM can be resolved using our experimental protocol, thus allowing for differences in σ_{NaCl} of 0.02 to be distinguished. Furthermore, the Stern-Volmer relation for SPQ quenching by chloride is unaffected by SPQ concentration between 0.5 and 20 mM and therefore is unaffected by the rapid volume change which occurs with the hyposmotic shock.

Under the conditions of these experiments, it is unlikely that the movement of either Na or Cl would be affected by solvent drag without also affecting the counter ion. The experiments were done in the nominal absence of CO_2 and without pH gradients (thus minimizing the effect of co- and countertransport systems such as the Cl-HCO_3 exchanger and the Na-H antiporter). More importantly, water flux occurs with a time constant of <150 ms that is more than fifty times faster than carrier mediated ion transport; any rapid early Na and Cl fluxes seen would be due to solvent drag. It should be noted, however, that if a solvent drag effect had been seen, this would not necessarily have implied that both Na and Cl were entering the water channel. It would be possible for one ion to be "dragged" through the water channel while the other followed a separate conductive pathway, its movement augmented by the streaming potential. The absence of solvent drag seen in this study similarly implies that at least one of the two ions can not enter the channel.

Welling et al. (1987) recently reported a value of 0.5 for σ_{NaCl} in the intact proximal tubule basolateral membrane using a video technique to measure cell volume transients in response to a change in bath osmolality. The results reported here are not compatible with a basolateral membrane σ_{NaCl} of 0.5. The difference in the methods employed deserves specific comment. Our experiments were performed on vesicles isolated from proximal tubule cell membranes. Membrane vesicles have been used extensively to study transport characteristics of isolated brush border and basolateral plasma membranes. Previous studies have established the integrity of transport systems such as the Na/H antiporter (Ives et al., 1983) and the Na/glucose cotransporter (Kaunitz and Wright, 1984). It is still possible that low reflection coefficient pores are lost or rendered inactive in the preparation of vesicles. However, the P_f and activation energy found in these and in other vesicle experiments (Meyer and Verkman, 1987; Verkman et al., 1985; Jacobson et al., 1982) are comparable with those found in the intact tubule (when normalized by the geometric folding factor) (Andreoli and Schafer, 1978; Gonzalez et al., 1982), making it unlikely that the vesicles have lost or closed a significant number of water channels. We cannot rule out regulated alteration of the basolateral membrane's σ_{NaCl} upon changing the bathing medium in the intact tubule experiments. Events such as insertion or activation of low reflection coefficient pores might occur in the intact tubule but not in the vesicle experiments and result in a change in σ_{NaCl}. This question needs to be explored further by the application of our fluorescence method for detection of solvent drag to the intact tubule, particularly by use of newer indicators with improved Cl sensitivity (Verkman et al., 1989).

While vesicle studies provide a powerful tool for determination of isolated apical or basolateral membrane reflection coefficients,[2] the transepithelial σ_{NaCl} cannot be determined directly. The transcellular reflection coeffi-

[2]A distinct advantage of the vesicle system is the lack of an unstirred layer effect. Assuming a solute diffusion coefficient of 10^{-5} cm^2/s, diffusion through an unstirred layer as large as the vesicle itself (~0.2 μ) would occur in <10 μs, which is well below the dead time of the stopped flow apparatus (~2 ms).

cient can be obtained from the separate apical and basolateral reflection coefficients by use of the equation for the reflection coefficient for two membranes in series (Kedem and Katchalsky, 1963):

$$\sigma_{tc} = \sigma_{ap} P/P_{ap} + \sigma_{bl} P/P_{bl}, \quad (4)$$

where σ_{tc} = transcellular σ_{NaCl}, σ_{ap} = apical σ_{NaCl}, σ_{bl} = basolateral σ_{NaCl}, P_{ap} = apical membrane NaCl permeability, P_{bl} = basolateral NaCl permeability and $P = P_{ap}P_{bl}/(P_{ap} + P_{bl})$. Because this equation is a weighted average, when the reflection coefficients for the isolated membranes are equal, then the composite σ_{NaCl} is equal to σ_{NaCl} for the individual membranes. Thus, using this equation with the lower limits of our estimates for the respective values for σ_{NaCl}, the transcellular reflection coefficient also has a lower limit of 0.92 by the induced osmosis method and 0.98 by the solvent drag method (with a best estimate of 1.0). If the predominant pathway for water movement in the proximal tubule is transcellular, then the transepithelial σ_{NaCl} is approximately equal to the transcellular σ_{NaCl}. The contribution of the paracellular pathway to volume reabsorption in the proximal tubule is uncertain although several studies indicate that it is small (Preisig and Berry, 1985).

One proposed role for a low transepithelial σ_{NaCl} is in the mechanism of isosmotic volume reabsorption. Luminal fluid can be made effectively hypotonic if high reflection coefficient solutes are preferentially reabsorbed in the early proximal tubule leaving low reflection coefficient solutes in the luminal fluid. An effective osmotic gradient from interstitium to lumen would result, even in the absence of a cryoscopic osmotic gradient because of the differences in reflection coefficients for the luminal and interstitial solutes. Thus, osmotic water movement would occur in the absence of a measurable osmotic gradient.

However, effective luminal hypotonicity cannot develop with a transcellular σ_{NaCl} of one unless the paracellular pathway makes a significant contribution to volume flux. However, it is unnecessary to invoke effective luminal hypotonicity to account for the (nearly) isosmotic volume reabsorption in the proximal tubule. Small degrees of measurable luminal hypotonicity (from 2 to 8 mOsm) have been found in this nephron segment (Green and Giebisch, 1984; Liu et al., 1984). Taken together with a very high P_f (Andreoli and Schafer, 1978; Gonzalez et al., 1982; Berry and Verkman, 1988) and possibly a small standing gradient in the cortical interstitium (Williams and Schafer, 1988), this osmotic gradient is sufficient to account for near isosmotic volume reabsorption (Berry, 1983). A high transepithelial σ_{NaCl} also rules out a solvent drag contribution to NaCl reabsorption.

Another proposed role for a low σ_{NaCl} is in mediating the volume regulatory decrease seen when the isolated lumen-collapsed proximal tubule is exposed to a hyposmotic shock under conditions of inhibited active transport (so that the cells are at Donnan equilibrium). In the model of the heteroporous membrane reported by Welling and Welling (1988), it was suggested that there are two populations of pores in the basolateral membrane. Both types of pores have a high reflection coefficient for the major intracellular solutes but one type has a low σ_{NaCl} (0.3) while the other has a high σ_{NaCl} (1.0). The membrane has a mean σ_{NaCl} of 0.65. In their model, the cell swells after a hyposmotic shock and then volume regulates by allowing solvent and NaCl to exit through the low σ_{NaCl} pores. At Donnan equilibrium, when intra- and extracellular [NaCl] are both high, there is no osmotic gradient across either pore and therefore zero volume movement until the cell is exposed to the osmotic shock. Under these conditions the heteroporous membrane provides a good model for cell volume regulation. However, under physiologic conditions the extracellular fluid is predominantly NaCl which has a low reflection coefficient for one of the pore types while the intracellular fluid is predominantly composed of solutes with a high reflection coefficient for both pores. This creates an outwardly directed effective osmotic gradient across the low σ_{NaCl} pore resulting in osmotic water influx which, at steady state, must be balanced by efflux through the high reflection coefficient pores. Thus, there is a constant circulating volume movement. Volume influx through the low σ_{NaCl} pores would deliver (by solvent drag) an enormous quantity of NaCl into the cell. Using average reported values for proximal tubule P_f and surface area, in conjunction with the values used by Welling et al. for the pores (1.0 for the high σ pore and 0.3 for the low σ pore) the solvent drag mediated Na and Cl influx through the low reflection coefficient pore would be ~670 pmol/mm-min. This would exceed the Na transport capacity of the Na, K-ATPase which has been estimated at 30 to 75 pmol/mm-min (Jorgensen, 1980).

The heteroporous membrane provides an elegant mechanism for cell volume regulation at Donnan equilibrium but would provide a large energetic burden on the cell under physiologic conditions. There are other mechanisms that can account for passive volume regulation. An osmo-receptor coupled to nonspecific ion channels or carriers provide one possible mechanism. Another, which has an increasing body of literature supporting it, uses stretch activated channels as a mechanism for passive solute exit from cells exposed to a hyposmotic shock and thus would allow for cell volume regulation under both physiologic conditions and those in which active transport is inhibited. These have been demonstrated in a number of cell types including proximal tubule cells (Sackin, 1987).

Theoretical considerations also require approximately equal apical and basolateral membrane reflection coefficients. An "isosmotically" reabsorbing epithelium (where the cryoscopic osmolality of the cortical interstitium is approximately equal to the luminal fluid) with an apical membrane σ_{NaCl} of 1 and basolateral σ_{NaCl} of 0.5 would be in a state of constant water secretion. If these values are reversed (apical 0.5 and basolateral 1), the cell moves water in the right direction but at a rate of 12 nl/mm-min (unless extremely high interstitial pressures are maintained). This far exceeds the maximal values of 2 nl/mm-min measured in the rabbit PCT (Berry, 1983).

The results of the current study for σ_{NaCl} and our recent finding in BBMV of unity reflection coefficient for urea (Chen et al., 1988*b*), suggest that the proximal tubule water channels are highly selective. Viewed in conjunction with previously reported values for P_f/P_d, this high selectivity has implications for the physical properties of the water channel. The interpretation of P_f/P_d depends on the physical characteristics of the pore (Finkelstein, 1987). For a "macroscopic pore" (in which bulk water movement can occur), (P_f/P_{d-1}) is proportional to the square of the pore radius (Solomon, 1968). The P_f/P_d ratio has been measured in the proximal tubule basolateral membrane ($P_f/P_d = 10$; Verkman and Wong, 1987) and in other biological membranes thought to contain aqueous channels including human erythrocyte and vasopressin stimulated toad bladder. A P_f/P_d of 10 would give a pore radius of ~11 Å which would admit small solutes and is therefore inconsistent with our findings for σ_{NaCl} as well as with the lack of solvent drag found by Corman and Di Stefano (Corman and Di Stefano, 1983) and by Jacobson et al. (1982) in the intact tubule. On the other hand, for a single file pore P_f/P_d is equal to the number of water molecules in the pore (Finkelstein, 1987). Taken together, the high selectivity and high P_f/P_d ratio suggest that, at least for the basolateral membrane, water channels are single file pores ~10 water molecules in length (giving a length of ~30–35 Å) and exclude all solutes.

In view of the above experimental findings and theoretical considerations we conclude that apical and basolateral membrane NaCl reflection coefficients are near unity and that a low σ_{NaCl} for either membrane would disrupt important cell functions. In the apical membrane a low σ_{NaCl} would act to dissipate ion gradients used to power secondary active transport systems such as Na-glucose and Na-amino acid co-transport and Na/H exchange. A low basolateral σ_{NaCl} without a low apical σ_{NaCl} would require the cell to be in a constant state of water secretion. Furthermore, a low basolateral σ_{NaCl} (as a component of a heteroporous membrane) would act to drag in large quantities of Na and Cl thus forcing upon the cell a large metabolic burden. The physiologic functions of the proximal tubule are best served by a single file pore which has high osmotic water permeability but excludes all solutes by steric hindrance.

Dr. Verkman is an established investigator of the American Heart Association.

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