PROTON NUCLEAR MAGNETIC RESONANCE MEASUREMENT OF DIFFUSIONAL WATER PERMEABILITY IN SUSPENDED RENAL PROXIMAL TUBULES

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ABSTRACT Diffusional water permeability was measured in renal proximal tubule cell membranes by pulsed nuclear magnetic resonance using proton spin-lattice relaxation times (T_1). A suspension of viable proximal tubules was prepared from rabbit renal cortex by Dounce homogenization and differential seiving. T_1 measured in a tubule suspension (22% of exchangable water in the intracellular compartment) containing 20 mM extracellular $MnCl_2$ was biexponential with time constants 1.8 ± 0.1 ms and 8.3 ± 0.2 ms (mean ± SD, n = 8, 37°C, 10 MHz). The slower time constant, representing diffusional exchange of water between intracellular and extracellular compartments, increased to 11.6 ± 0.6 ms (n = 6) after incubation of tubules with 5 mM parachloromercuribenzene sulfonate (pCMBS) for 60 min at 4°C and was temperature dependent with activation energy E_a = 2.9 ± 0.4 kcal/mol. To relate T_1 data to cell membrane diffusional water permeabilities (P_d), a three-compartment exchange model was developed that included intrinsic decay of proton magnetization in each compartment and apical and basolateral membrane water transport. The model predicted that the slow T_1 was relatively insensitive to apical membrane P_d because of low luminal/cell volume ratio. Based on this analysis, basolateral P_d (corrected for basolateral membrane surface convolutions) is 2.0×10^{-3} cm/s, much lower than corresponding values for basolateral P_f ($10–30 \times 10^{-3}$ cm/s) measured in the intact tubule and in isolated basolateral membrane vesicles. The measured $P_f/P_d > 1$, low E_a and inhibition of P_d by pCMBS provide strong evidence that water transport in the proximal tubule basolateral membrane is facilitated by a specialized aqueous pore or narrow channel.

INTRODUCTION

The renal proximal tubule consists of a single layer of polarized epithelial cells containing both transcellular and paracellular pathways for water and solute movement. In response to a small transepithelial osmotic gradient, there is very rapid transcellular osmotic water flow, which consists of serial movement of water across the apical (luminal) membrane, cell cytoplasm, and basolateral membrane. Proximal tubule osmotic water permeabilities (P_f) have been characterized in the intact proximal tubule (for review see Berry, 1983), in isolated apical and basolateral membrane vesicles (Verkman et al., 1985; Verkman and Ives, 1986), and in isolated proximal tubule cells (Meyer and Verkman, 1987). Transepithelial P_f is high (~0.2 cm/s calculated for a cylindrical smooth tubule, ~0.01 cm/s when expressed per unit area of unfolded membranes),[1] with correspondingly high basolateral > apical membrane water permeabilities.

The diffusional water permeability (P_d) of the proximal tubule has been more difficult to measure. A lower limit to transepithelial P_d has been estimated from tracer flux studies to be 12.5×10^{-3} cm/s (calculated for a cylindrical smooth tubule) using measured butanol flux to estimate a correction factor for unstirred layer effects (Berry, 1985). For a transepithelial P_f of 0.2 cm/s, P_f/P_d is ~16 suggesting either that there are large pores in proximal tubule cell membranes, or that unstirred layer effects have not been adequately accounted for in P_d determinations. Because of rapid water exchange times, there have been no reported

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[1] In whole tubule physiology, transepithelial fluxes are frequently expressed per unit apical membrane area of a hypothetical right cylindrical surface. Because both the apical and basolateral membranes are highly convoluted (Welling and Welling, 1975), fluxes and permeability coefficients are lower when expressed per unit area of unfolded membranes.

measurements of P_d across individual apical or basolateral membranes.

We report here the application of proton nuclear magnetic resonance (NMR) to measure P_d across proximal tubule cell membranes using T_1 measurements in intact viable proximal convoluted tubules in suspension. The purpose of these studies is to determine cell membrane P_d (and thus P_f/P_d), its activation energy (E_a), and the effect of the organic mercurial parachloromercuribenzene sulfonate (pCMBS). Based on a three-compartment mathematical model of decaying and exchanging proton magnetization in suspended tubules, it is concluded that T_1 NMR measurements are sensitive to and thus measure basolateral rather than apical P_d. We find that basolateral membrane diffusional water transport is 60% inhibitable by pCMBS with low E_a = 2.9 kcal/mol, and that basolateral membrane P_f/P_d is ~10, which is consistent with the presence of a facilitated water transport pathway in the proximal tubule basolateral membrane.

METHODS

Materials

$^{54}MnCl_2$ and ^{3}H-inulin were obtained from New England Nuclear (Boston, MA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Magnetic iron oxide was prepared by the method of Cook and Pickering (1958).

Proximal Convoluted Tubule Isolation

Suspensions of proximal convoluted tubules were prepared by modification of the techniques of Chung et al. (1982) and Sakharani et al. (1984) without collagenase digestion. One or two New Zealand white rabbits (female, 2–3 kg) were sacrificed by decapitation and the renal arteries were cannulated and perfused with buffer A (modified Hank's media, composition in millimolars: NaCl, 137; KCl, 3; $CaCl_2$, 2; $MgCl_2$, 0.5; $MgSO_4$, 0.4; KH_2PO_4, 0.5; Na_2HPO_4, 0.3; D-glucose, 5; L-lactate, 4; *L*-alanine, 1; bovine serum albumin, 0.2% wt/vol; titrated to pH 7.4 with Tris, 4°C) until blanching of the kidney occurred. An additional 2 ml of 0.5% solution of iron oxide in solution A was infused until the kidney became a homogeneous grey. The kidneys were removed and the grey cortex was dissected in long strips away from the pale medulla and placed in buffer A at 4°C. The strips were homogenized in a 7-ml Dounce B homogenizer (Wheaton Instruments Div.; Wheaton Industries, Millville, NJ) (5 strokes). The homogenate was sequentially filtered through 250 and 70-μm nylon meshes (Small Parts, Inc., Miami, FL), retaining proximal convoluted tubules (PCT) on the 74-μm mesh. This step was repeated three to four times until no further tubules were retained. Proximal convoluted tubule-enriched material trapped on the 74-μm mesh was resuspended in buffer A. The glomeruli containing iron oxide were removed by a magnetic stirring bar. The suspension contained >95% PCT as judged by light microscopy.

Tubules were centrifuged at low speed (200 *g* for 2 min) and washed once in 50 vol of 150 mM NaCl, 5 mM HEPES, pH 7.4 (buffer B). The tubule pellet (~1.5 ml/kidney) was maintained at 4°C until the time of the experiment. Tubule viability was confirmed by trypan blue exclusion and O_2 uptake as described elsewhere (Soltoff and Mandel, 1984). In some experiments, pCMBS was added to the tubule pellet (diluted 20% with buffer B) from a freshly prepared 10-mM solution in buffer B and incubated for 60 min in the dark at 4°C. Tubules were heated to 8°, 25°, or 37°C 3 min before the NMR measurement. Just before the NMR measurement, $MnCl_2$ was added from a 0.5-M aqueous solution to give an extracellular Mn concentration of 20 mM. Unless otherwise specified, NMR measurements were performed within 3 min after Mn addition at which time tubules were fully viable and contained no Mn.

Nuclear Magnetic Resonance Measurements

P_d was measured using proton spin-lattice relaxation times (T_1) based on techniques developed for red blood cells (Conlon and Outhred, 1972; Fabry and Eisenstadt, 1978) and applied recently to intact gallbladder epithelia (Steward and Garson, 1985). T_1 was measured using a 90°-τ-90° pulse sequence using a proton NMR instrument (Praxis II model PR1005; Praxis Corp., San Antonio, TX) operating at 10 MHz. The amplitude of the free induction decay following the second 90° pulse (M_z, the z-component of residual magnetization) was determined for 32 τ values (0–16 ms). Each free induction decay was averaged 20 times to improve signal-to-noise ratio. M_z decayed with increasing τ according to biexponential kinetics,

$$M_o - M_z = A_1 \exp(-\tau/a_1) + A_2 \exp(-\tau/a_2), \quad (1)$$

where M_o is the equilibrium value of magnetization, A_1 and A_2 are related to the total number of extracellular and intracellular protons, respectively, and a_1 and a_2 are apparent T_1 relaxation times. As will be shown in the Results section, a_2 is an apparent exchange time that is related to cell membrane P_d.

^{54}Mn Uptake Measurements

A filtration assay of ^{54}Mn uptake was performed to determine whether cell uptake of extracellular Mn occurred over the time course of the NMR studies (Dix and Solomon, 1984). 0.2 ml of the packed tubule pellet was suspended in 2 ml of solution containing 150 mM NaCl, 5 mM HEPES, 20 mM $MnCl_2$, 11.4 μCi $^{54}MnCl_2$, pH 7.4 at 37°C. At various times, 100-μl samples were removed, filtered on 8-μm filters (model HAWP; Millipore Corp., Bedford, MA) and washed three times with 2 ml of buffer B containing 20 mM $MnCl_2$ at 4°C. Radioactivity was measured in a gamma counter (Nuclear Chicago; TM Analytic, Elk Grove Village, IL). Radioactivity measured in a 0 time sample was subtracted from each value to correct for ^{54}Mn binding to the tubule surface (<10% of equilibrium radioactivity). Corrected cpm (expressed as % of equilibrium uptake radioactivity) at 1, 2, 3, 4, 5, 10, 15, 20, 30, and 60 min incubations were −0.1, 0.3, 0.9, 1.4, 0.4, −0.2, 7.2, 11, 21, and 26%, respectively (SD, ~0.3%), indicating no measureable uptake of ^{54}Mn over 10 min at 37°C.

RESULTS

Spin-lattice relaxation times for tubule suspensions are shown in Fig. 1. For a packed suspension of tubules in the absence of Mn, a single T_1 of 770 ± 40 ms (mean ± SD, n = 5) was measured, similar to T_1 values of 500–1,000 ms reported for cytoplasmic water protons in several cell systems (Fabry and Eisenstadt, 1978; Steward and Garson, 1985). In the presence of tubules suspended in buffer containing 20 mM $MnCl_2$ (curve labeled −pCMBS) the magnetization decay was biexponential with time constants of 1.78 and 8.34 ms. The data shown are the average of magnetization decays obtained from eight separate tubule preparations. The average T_1's from biexponential analyses performed on each tubule sample were 1.7 ± 0.1 ms and 8.3 ± 0.3 ms (mean ± SD), indicating little biological variability in measured T_1 values.

pCMBS is an inhibitor of human erythrocyte water transport. After a 60-min incubation, 2.5 mM pCMBS

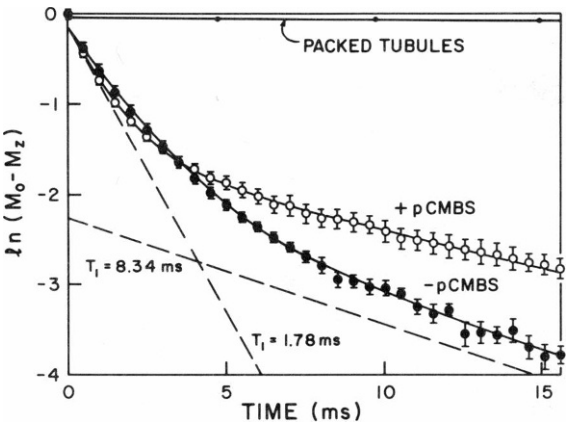

FIGURE 1 Proton spin-lattice relaxation times of tubule suspensions. The ordinate is the ln (magnetization) (Eq. 1) measured by a 90°-τ-90° pulse sequence as described in Methods. The abcissa is the time interval between the 90° pulses. Measurements performed at 37°C. Packed tubule suspension in buffer B in the absence Mn with T_1 = 762 ms. Only the first four data points are shown. Solutions for the curved plots consisted of tubules (~20% of total exchangeable water intracellular) in buffer B containing 20 mM extracellular $MnCl_2$ incubated with 0 or 5 mM pCMBS for 60 min at 4°C. Each data point is the mean ± SD of measurements performed using eight (−pCMBS) and six (+pCMBS) separate tubule preparations. Data fitted to a biexponential function (Eq. 1) with a_1 = 1.78 ms, a_2 = 8.34 ms, and A_1/A_2 = 2.5 (−pCMBS), and a_1 = 1.20 ms, a_2 = 11.6 ms, and A_1/A_2 = 2.9 (+pCMBS). The individual exponential decay processes for the −pCMBS curve are indicated.

inhibits erythrocyte P_f by 90% and P_d by 50% (Macey and Farmer, 1970). Incubation of suspended tubules with 5 mM pCMBS for 60 min at 4°C resulted in a 36% increase in the slower T_1 (curve labeled +pCMBS). The average T_1's from biexponential analyses performed on six separate tubule preparations were 1.3 ± 0.1 ms and 11.7 ± 0.7 ms (SD). In paired studies in five separate tubule preparations, 5 mM pCMBS caused increases in T_1 by 32, 17, 43, 33, and 24%. As discussed below, prolongation of T_1 corresponds to inhibition of tubule cell membrane diffusional water permeability. In three experiments in which T_1 was measured within 5 min after pCMBS addition there was no significant prolongation in T_1 (6 ± 4%). In two experiments in which the pore-forming antibiotic amphotericin (4 mg/ml packed tubules) was added immediately before the NMR measurement, T_1 decreased to 5.1 ± 0.3 ms.

In buffer B containing 10, 20, 30, 40, and 50 mM $MnCl_2$ in the absence of tubules, T_1 was 7.1, 3.6, 2.4, 1.7, and 1.4 ms, respectively (for each value SD <0.2 ms, n = 3). Interestingly, the faster T_1 (a_1) of the biexponential decay (1.78 ms), representing proton decay in extracellular solution is smaller than the corresponding T_1 (3.6 ms) measured in buffer B. This effect is probably caused by two physical processes. The faster T_1 is a composite apparent time constant that depends upon the rates of intrinsic proton decay and proton exchange between the tubule cell and the extracellular compartment and the relative sizes of each compartment. Using the eigenvalue analysis presented below, 10–15% of the decrease in the intrinsic extracellular proton T_1 can be accounted for (3.6 to ~3.2 ms). More importantly, it is known that albumin and other Mn-binding proteins cause enhanced quenching efficacy of Mn. Because the dense tubule suspension probably contains proteins that adhered to the tubules during isolation or that were released from nonviable tubules, it is likely that the intrinsic decay rate of extracellular protons is greater than that measured in a protein-free buffer.

It is generally found that Mn permeates cell membranes very slowly and does not itself alter water permeability. These assumptions were tested in the suspended proximal tubule system. T_1 was measured 0, 1, 2, 3, 5, 10, and 15 min after addition of 20 mM $MnCl_2$ and incubations of tubules at 37°C. The slower T_1 values (a_2) were 8.2, 8.4, 8.2 8.0, 7.8, 7.6, and 5.1 ms, respectively, indicating no measureable entry of Mn into the cells at 5 min of incubation at 37°C. This finding was confirmed by direct ^{54}Mn-uptake measurements as described in Methods. Similar NMR experiments performed in tubules incubated with pCMBS showed no entry of Mn at 4 min. As Mn enters tubule cells, there is a decrease in amplitude and time constant for the slower T_1 decay process due to an increased intrinsic decay rate of intracellular protons. Addition of Triton X-100 to the tubule suspension, which solubilizes the membrane, resulted in a single T_1 of 1.5 ms. It is more difficult to show that Mn does not alter P_d because P_d cannot be measured in the absence of Mn. Table I shows that P_d is not altered by Mn over the concentration range of 10–30 mM, however, it is not possible to exclude an effect of <10 mM Mn on water permeability.

The effect of temperature on diffusional water transport was studied to determine the activation energy (E_a) for P_d (Fig. 2). With increasing temperature, there was a decrease in the slower T_1 (a_2) from 13.7 ms (8°C) to 8.5 ms (37°C), which corresponds to increasing diffusional water

TABLE I
DEPENDENCE OF DIFFUSIONAL WATER PERMEABILITY ON [Mn]

[Mn]	a_1	a_2	$A_2/(A_1 + A_2)$	P_d^{bl}
mM	*ms*	*ms*		*cm/s* × 10^{-3}
5	5.5	30.3	0.18	—
10	2.5	8.6	0.31	2.18
20	1.5	8.7	0.13	1.76
30	1.3	8.1	0.08	1.89
40	0.9	5.3	0.09	3.31

Magnetization decays were fitted to the biexponential function in Eq. 1 for increasing $MnCl_2$ concentrations. P_d^{bl} was calculated according to the method given in Eq. 3 and in Fig. 4 with $k_2 = 1/a_1$, $k_1 = k_3 = 1.3\ s^{-1}$, $(S/V)_{ap} = (S/V)_{bl} = 41{,}000\ cm^{-1}$, $V_2/V_3 = 0.2$ and $V_1/V_3 = 6$. Results represent one set of experiments typical of two. Errors in P_d measurements are ~0.2×10^{-3} cm/s.

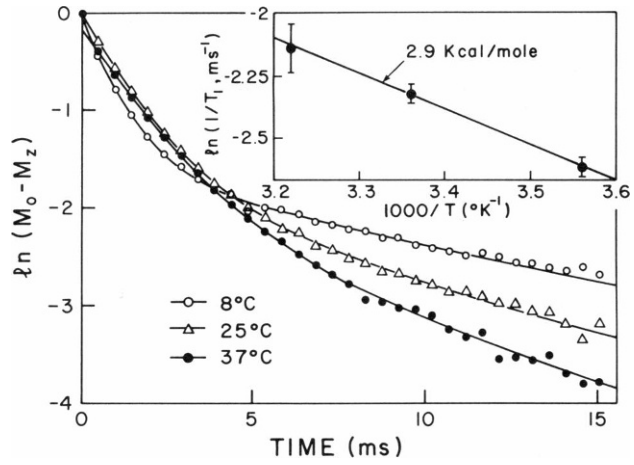

FIGURE 2 Temperature dependence of tubule P_d. Proton spin-lattice relaxation was measured at varying temperatures under conditions identical to those in Fig. 1 in the absence of pCMBS. Data from four to eight individual tubule preparations were averaged for each curve. Parameters for biexponential fits were: at 8°C, a_1 = 0.93 ms, a_2 = 21 ms, and A_1/A_2 = 5.14; at 25°C, a_1 = 1.29 ms, a_2 = 13.5 ms, and A_1/A_2 = 4.63; at 37°C, same fit used in Fig. 1. *Inset,* Arrhenius plot of ln ($1/T_1$ = $1/a_2$) vs. temperature^{-1} with fitted activation energy of 2.9 ± 0.4 kcal/mol.

permeability. Fig. 2, *inset,* shows an Arrhenius plot of ln $1/T_1$ vs. temperature^{-1}, with E_a = 2.9 ± 0.4 kcal/mol. This value is a model-independent estimate of E_a because it is assumed that cell membrane P_d is proportional to $1/T_1$. A more exact approach to determine P_d is presented below. E_a would increase to 4 kcal/mol if calculated from the slope of a ln P_d vs. temperature^{-1} plot.

Calculation of P_d from T_1 Relaxation Times

Estimation of absolute cell membrane P_d requires knowledge of tubule geometry. As shown in Fig. 3, in an idealized manner, the renal proximal tubule consists of a cylindrical layer of epithelial cells joined at the apical (luminal) membrane by junctional complexes. The cell basolateral membrane covers the cell surfaces adjacent to the lateral intercellular space and to a highly permeable basement membrane that wraps around the proximal tubule. Because of the uneven distribution of basolateral membrane surface convolutions, 60–80% of the total basolateral surface faces the basement membrane (Welling and Welling, 1986).

The decay kinetics of proton magnetization is predicted uniquely from apical and basolateral membrane P_d's, the rates of intrinsic proton decay in each compartment, and the detailed tubule geometry. Because of uncertainties in many of these parameters, a simplified three-compartment model of exchanging spins will be adopted (Fig. 3). Each compartment i has volume V_i and a spontaneous rate of proton decay by T_1 relaxation of k_i. $k_{i,j}$ is the exchange rate constant for proton movement from compartments i to j.

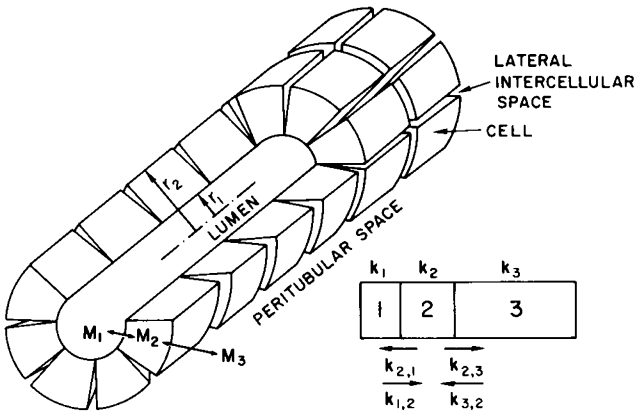

FIGURE 3 Cutaway schematic section of a proximal tubule showing luminal, cell, and extracellular compartments with simplified three-compartment model. Definition of parameters: r_1, internal tubule radius; r_2, external tubule radius; M_i, magnetization in the ith compartment; k_i, intrinsic decay rate for proton magnetization in the ith compartment; $k_{i,j}$, exchange rate for proton movement from compartments i to j.

The decay of magnetization in the ith compartment is described by

$$dM_i/dt = k_{i-1,i}M_{i-1} - (k_{i,i-1} + k_i + k_{i,i+1})M_i + k_{i+1,i}M_{i+1}, \quad (2)$$

where M_i is the deviation of magnetization from its equilibrium value in the ith compartment. Because net water flux across each membrane is zero at equilibrium, $k_{i,j}V_i = k_{j,i}V_j$, where V_i is assumed to be proportional to the number of exchangeable protons. $k_{2,1}$ and $k_{2,3}$ are related to apical and basolateral P_d(P_d^{ap} and P_d^{bl}) by the relations $k_{2,1} = P_d^{ap}(S/V)_{ap}$ and $k_{2,3} = P_d^{bl}(S/V)_{bl}$, where $(S/V)_{ap}$ and $(S/V)_{bl}$ are the apical and basolateral membrane surface-to-volume ratios, respectively. The coupled linear differential equations describing the three-compartment model can be written in matrix form,

$$\frac{dM}{dt} = \begin{pmatrix} -k_1 - k_{2,1}V_2/V_1 & k_{2,1} & 0 \\ k_{2,1}V_2/V_1 & -k_2 - k_{2,1} - k_{2,3} & k_{2,3}V_2/V_3 \\ 0 & k_{3,2} & -k_3 - k_{3,2}V_2/V_3 \end{pmatrix} M, \quad (3)$$

where M is the magnetization vector (M_1, M_2, M_3). The eigenvalues of the matrix are the apparent T_1 relaxation times for the triple exponential decay of magnetization and the eigenvectors are related to the relative amplitude of each decay process. For the suspended tubule experiment, two of the three eigenvalues would be large and correspond to the rapid-measured T_1 of ~1.8 ms. The third, small eigenvalue is the reciprocal of the slow-measured T_1 (a_2).

Based on this formulation, the predicted dependences of the slow T_1 on P_d^{ap} and P_d^{bl} are shown in Fig. 4. The parameters for the simulation were chosen as follows: k_1 =

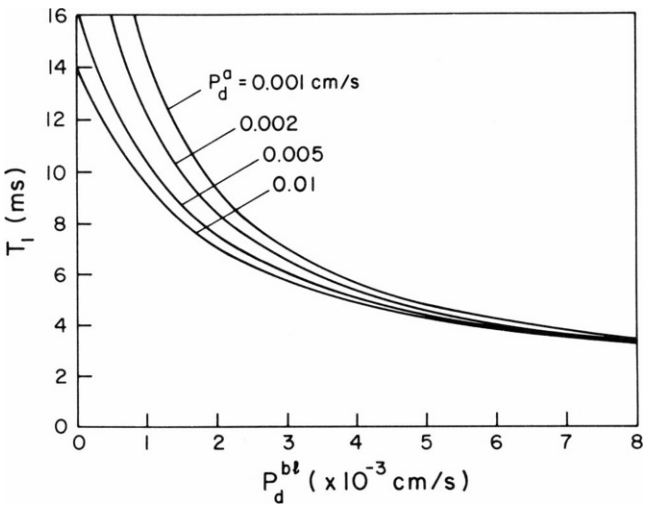

FIGURE 4 Dependence of slow T_1 (a_2) on apical and basolateral membrane water permeabilities. T_1 was calculated from the reciprocal of the smallest eigenvalue for the matrix in Eq. 3 as described in the text. P_d^{ap} and P_d^{bl} were varied. Parameters: $k_1 = k_3 = 556\ s^{-1}$, $k_2 = 1.3\ s^{-1}$, $(S/V)_{ap} = (S/V)_{bl} = 41{,}000\ cm^{-1}$, $V_2/V_3 = 0.2$, and $V_2/V_1 = 6$.

$k_3 = 556\ s^{-1}$ calculated from measured $1/a_1$. $k_2 = 1.3\ s^{-1}$ from T_1 measured in a packed tubule suspension. The calculations are very insensitive to changes in k_2. $(S/V)_{ap} = (S/V)_{bl} = 41{,}000\ cm^{-1}$ was taken from morphometric analyses of Welling and Welling (1975). $V_2/V_3 = 0.2$ was determined experimentally. 2 μCi of ^{3}H-inulin was added to a 50% dilution of packed tubules in buffer B. The ratio of radioactive counts per unit volume in the mixture (9,680 cpm) to that in the supernatant after centrifugation (20,800 cpm) gave a fractional volume occupied by tubules of 0.32 ± 0.02 (SD, $n = 2$) in a packed tubule suspension. Because tubules are diluted 1.2-fold for NMR measurements and because ~15% of tubule cell volume is composed of nonaqueous solids (Meyer and Verkman, 1986), intracellular exchangeable water comprises ~22% of total volume. $V_2/V_1 = 6$ based on tubule dimensions ($r_1 = 11\ \mu$, $r_2 = 22\ \mu$m), and assuming that the average tubule is half-distended.

The data in Fig. 4 indicate that T_1 is much more sensitive to P_d^{bl} than to P_d^{ap}. When $P_d^{ap} = P_d^{bl} = 2 \times 10^{-3}$ cm/s, $T_1 = 8.36$ ms. A 50% increase in P_d^{bl} would decrease T_1 by 23%, whereas a 50% increase in P_d^{ap} would decrease T_1 by 5%. For $T_1 = 8.33$ ms measured for the tubule suspension at 37°C in the absence of pCMBS, P_d^{bl} is 2.0×10^{-3} cm/s, assuming $P_d^{ap} = 2 \times 10^{-3}$ cm/s. As the estimated value for P_d^{ap} varied 10-fold ($1–10 \times 10^{-3}$ cm/s), calculated P_d^{bl} varied from 1.4–2.3 $\times 10^{-3}$ cm/s. For $T_1 =$ 11.6 ms, measured in the presence of pCMBS (Fig. 1), P_d^{bl} would be 0.85×10^{-3} cm/s, assuming $P_d^{ap} = 2 \times 10^{-3}$ cm/s.

DISCUSSION

Measurement of cell membrane diffusional water transport in the intact proximal convoluted tubule presents a number of difficulties. Because of the rapid exchange time for labeled water between intracellular and extracellular compartments (~6 ms at 37°C), uptake studies using tritiated H_2O are not feasible. In addition, the presence of extracellular unstirred layers seriously restricts the use of nonsteady-state methods to measure P_d.

Measurement of spin-lattice relaxation times by nuclear magnetic resonance provides a method to measure proximal tubule cell P_d under steady-state conditions. Rapid exchange times are measurable. Extracellular unstirred layer effects are eliminated in T_1 NMR measurements because extracellular Mn is not consumed after quenching water protons that have diffused from the intracellular to the extracellular compartments. Because of the limited sensitivity of magnetic resonance methods, T_1 measurements are performed using a tubule suspension rather than on a single isolated tubule. Very pure suspensions of viable proximal tubules can be prepared by a simple homogenization and seiving procedure within 45 min of animal sacrifice. Studies on tubule suspensions offer the advantages of technical ease, averaging of water transport properties of many tubules, and the ability to study effects of temperature and transport inhibitors on water transport using parallel tubule samples taken from a single preparation. In addition, unlike the toad bladder and gallbladder epithelia, the isolated proximal tubule does not have a thick layer of adherent subepithelial tissue. Despite these advantages, however, there are a number of potential difficulties in the interpretation of the NMR results as detailed below.

Because of the intrinsic polarity of proximal tubule epithelial cells, the T_1 relaxation times contain composite information about apical and basolateral membrane diffusional water permeabilities. Fortunately, the simple three-compartment analysis presented in the Results section showed that the slower T_1 is very sensitive to basolateral membrane P_d, yet relatively insensitive to apical membrane P_d. Conceptually, a magnetically labeled proton diffusing from the intracellular to the luminal compartments has a high probability of reentering the intracellular compartment without being quenched by luminal Mn. T_1 becomes independent of apical membrane P_d when the tubule lumen is collapsed, or if Mn does not enter the lumen at the time of the NMR measurement. Because T_1 is predicted to be insensitive to apical membrane P_d under worst case conditions, when the tubule lumen is not collapsed and its assumed to contain the same Mn concentration as is present in the extratubular fluid (Fig. 4), it is concluded that T_1 is primarily a measure of basolateral membrane P_d.

Several properties of basolateral membrane diffusional water permeability can be examined without precise knowledge of tubule cell geometry. pCMBS, a mercurial sulfhydryl reagent that inhibits red cell P_f by 90% (Macey and Farmer, 1970), inhibited basolateral membrane P_d by 60% at 37°C. This finding is in agreement with the reported inhibition of basolateral membrane P_f by 80% in

isolated perfused rabbit proximal straight tubules (2.5 mM pCMBS, 37°C; Whittembury et al., 1984) and with the inhibition of transepithelial P_d by 32% in rabbit proximal convoluted tubules when 2 mM pCMBS was added to fluid bathing the basolateral membrane (39°C; Berry, 1985). In addition, Meyer and Verkman (1987) found recently that P_f in basolateral membrane vesicles isolated from rabbit renal cortex was inhibited 65% by 5 mM pCMBS and 75% by 300 μM $HgCl_2$ (K_I = 42 μM).

The activation energy (E_a) for basolateral membrane P_d was 2.9 ± 0.4 kcal/mol (8°–37°C), similar to values of 2.5 ± 1 kcal/mol for P_f measured in basolateral membrane vesicles isolated from rabbit renal cortex (12°–50°C; Verkman and Ives, 1986), and of 4.3 kcal/mol measured for transepithelial P_d in isolated rabbit proximal convoluted tubules (20°–40°C; Berry, 1985). These values differ from E_a measured for P_f in apical membrane vesicles and isolated proximal tubule cells (~13 kcal/mol, >30°C and 2 kcal/mol <33°C; Verkman et al., 1985; Meyer and Verkman, 1987) and for transepithelial P_f measured in the isolated rabbit proximal tubule (15–20 kcal/mol, 20°–40°C; C. Berry, unpublished data).

Determination of absolute cell membrane P_d (and thus, P_f/P_d) requires knowledge of tubule geometry. Because in vivo cell geometry is preserved in suspended tubules, the morphometric analyses of rabbit proximal tubule performed by Welling and Welling (1975, 1986) apply and were used to calculate absolute P_d (see Results). The best value for P_d^{bl} was 2.0×10^{-3} cm/s assuming apical and basolateral membrane P_d's were equal. In extreme limiting cases, where the tubule lumen is collapsed ($P_d^{ap} = 0$), and where P_d^{ap} is very large (0.01 cm/s), P_d^{bl} would be 3.1×10^{-3} cm/s and 1.4×10^{-3} cm/s, respectively. If the tubule were completely distended (rather than half-distended as used for the calculation in Fig. 3), P_d^{bl} would be 1.7×10^{-3} cm/s. P_d^{bl} would be underestimated if the effective basolateral surface area is less than that reported by Welling and Welling (4.1 μm^2 surface area/μm^3 cell volume). This might occur if water diffusing from the cell into the lateral intercellular space (LIS) was not effectively quenched by Mn. Even if the LIS were very narrow, P_d^{bl} would be underestimated by at most 40% since 60–80% of basolateral membrane surface area faces the basement membrane (Welling and Welling, 1986).

Although extracellular unstirred layers do not affect cell membrane P_d measured by T_1 relaxation times in the presence of extracellular Mn, intracellular unstirred layers could result in an underestimate in P_d. The measured proton exchange time consists of the composite time required for a water proton to move from its position within the cell to the inner membrane surface and then from the inner to outer membrane surface by transmembrane water diffusion. The former time can be estimated assuming a low intracellular water diffusion coefficient (D) of 5×10^{-6} cm^2/s, one-fourth of D in free water (Chang et al., 1975). For a spherical cell of radius r_o, the average time required to reach the inner cell membrane is $0.025r_o^2/D$ (Meyer and Verkman, 1987), which is 1.3 ms for a 10-μm diameter proximal tubule cell. Since the measured exchange time was ~8 ms, we would expect at most a 20% underestimate of true basolateral membrane P_d because of restricted intracellular water diffusion.

Recognizing these limitations in the analysis, it is useful to calculate P_f/P_d. Basolateral membrane P_f has been measured in rabbit basolateral membrane vesicles to be 3×10^{-2} cm/s, which corrects to a value of 0.4 cm/s when expressed per unit length of a cylindrical smooth tubule (Verkman and Ives, 1986). This value is in agreement with estimates of basolateral membrane P_f of 0.28–0.4 cm/s (37°C) in the intact rabbit proximal tubule using a rapid video recording technique (Welling et al., 1983; Whittembury et al., 1984). Using the present P_d value expressed per unit length of a cylindrical smooth tubule (0.04 cm/s), basolateral membrane P_f/P_d is ~10. $P_f/P_d > 1$ has been measured in other systems thought to contain aqueous pores or channels including the human erythrocyte (P_f/P_d = 3.5; Solomon et al., 1984; Macey, 1984) and the intact toad bladder under vasopressin stimulation (P_f/P_d = 14; Levine et al., 1984). The high P_f/P_d, taken together with the low E_a and pCMBS inhibition of basolateral membrane P_d, supports the notion that proximal tubule basolateral water transport occurs through specialized aqueous channels. This view is supported by stopped-flow light scattering measurements of P_f in rabbit basolateral membrane vesicles, where E_a increased from 3.6 to 7.6 kcal/mol with inhibition by $HgCl_2$ (Meyer and Verkman, 1987). The physical nature of the water channel, whether composed of proteins and/or lipids, remains the subject of further investigation.

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