Biochimica et Biophysica Acta, 1030 (1990) 203–210
Elsevier

BBAMEM 75075

Effects of dimethylsulfoxide and mercurial sulfhydryl reagents on water and solute permeability of rat kidney brush border membranes

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(Received 20 July 1990)

Key words: Water channel; Mercurial sulfhydryl reagent; Dimethylsulfoxide, Reflection coefficient; Brush border membrane; (Rat kidney)

The effects of dimethylsulfoxide, DMSO, and mercurial sulfhydryl reagents have been studied on water and small solute permeability of rat renal brush border membrane vesicles. Water and solute permeability was measured by mixing membrane vesicles with hypertonic solutions in a stopped-flow apparatus and following osmotically-induced changes in vesicular volume via changes in scattered light intensity. The rate constant of the fast osmotic shrinkage is proportional to the osmotic water permeability, while the rate constant of the slow reswelling phase is proportional to the solute permeability. Using mannitol as the osmotic agent, the osmotic shrinkage of rat renal brush border membrane vesicles followed a biphasic time course. 80% of the vesicles shrunk with a rate constant of approx. 50 s^{-1} and 20% with a rate constant of approx. 2 s^{-1}. DMSO decreased dose-dependently the amplitude of the fast osmotic shrinkage, without affecting its rate constant. In contrast to DMSO, $HgCl_2$ decreased the rate constant but not the amplitude of the fast osmotic shrinkage of renal brush border vesicles. Between 40–50 μM $HgCl_2$, the inhibition of the fast osmotic shrinkage was completed. DMSO and $HgCl_2$ increase the activation energy of water permeation in renal membranes from 3 to 12–15 kcal/mol. DMSO and $HgCl_2$ did not affect the rate constant of the slow osmotic shrinkage of renal membrane vesicles and were also without effect on osmotic shrinkage of small intestinal brush border and pure phospholipid vesicles. In renal brush border membranes, $HgCl_2$ at low concentrations (< 10 μM) increased by 15-fold the permeability to NaCl and urea but not to mannitol, an effect which precedes the inhibition of water permeability at higher $HgCl_2$ concentrations. The increase in small solute permeability was irreversible while the inhibition of water permeability could be reversed with cysteine and dithiothreitol. We conclude that water and small solute pathways in rat renal brush border membranes are completely separate entities, which are effected differently by DMSO and $HgCl_2$. These pathways for water and solutes must be membrane proteins since neither DMSO nor $HgCl_2$ affect the permeability properties of pure phospholipid vesicles.

Introduction

Water movement across cell membranes is of central importance for living cells. For most cell membranes, diffusion of water through the lipid matrix is sufficiently rapid for proper cell function. A lipid-mediated diffusion mechanism for water permeation is characterized by strong hydrophobic interactions with high values for apparent activation energies, typically ranging between 10 to 16 kcal/mol [1–4]. For some specialized cell functions, water diffusion may not be adequate and evidence for water movement through specialized structures or channels has been provided [1–3]. The presence of channels or pores increases the velocity of water flow because they permit bulk flow or viscous flow with apparent activation energies close to 4 kcal/mol as can be expected for free solution viscous flow [2–10]. Our knowledge about the nature of water channels is at the moment rather superficial, which is, among other reasons, due to the fact that good specific inhibitors are not available. So far mercurial sulfhydryl reagents are the only known inhibitors of water channels [3,4,7,10–16].

Abbreviations: pCMBS, *p*-chloromercuribenzenesulfonic acid; Hepes, 4-(2-hydromethyl)-1-piperazineethanesulfonic acid; DMSO, dimethylsulfoxide.

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The brush border and the basolateral plasma membrane of proximal tubular cells from the kidney most likely contain water channels, since the observed values for osmotic water permeability are the highest reported for plasma membranes [4,7,11], the activation energy for osmotic water permeation is very low [4,10] and mercurial sulfhydryl reagents reduce water permeability coefficients by one order of magnitude [4,7,11,13]. Since inhibitors of water movement and substances which can dissociate water from solute movements can be important tools in studying the nature of water transport pathways, we have used brush border membrane vesicles from rat renal cortex to study in more detail the effects of dimethylsulfoxide, DMSO, and mercurial sulfhydryl reagents on water and solute permeation.

With DMSO as well as with $HgCl_2$ we were able to uncouple water and solute permeation through renal plasma membrane vesicles, which implies that water and solute pathways are separate entities. In addition, the results of this study strongly support the contention that effects of mercurial sulfhydryl reagents on water permeability of renal brush border membranes are protein-related phenomena.

Methods and Materials

Membrane preparations

Brush border membranes of rat small intestine were isolated according to Hauser et al. [17]. Rat renal brush border membranes were isolated as described by Biber et al. [18]. The membrane vesicle preparations were washed twice in 50 mM mannitol, 2 mM $MgCl_2$ and 5 mM Hepes-Tris (pH 7.4) and were used either freshly or after storage in liquid N_2 for up to 2 weeks. No effects of storing could be detected on water and solute (mannitol, NaCl, urea) permeabilities of membrane vesicles. Enzymes and protein were assayed as before [4]. Purification factors for the relevant marker enzymes were as previously reported [4]. In control studies with pure lipid membrane vesicles, 100 mg asolectin was sonified in 2.5 ml solution containing 50 mM mannitol, 2 mM $MgCl_2$ and 5 mM Hepes-Tris (pH 7.4) for 4×30 s with a Branson sonifier at maximum output (100 W).

Water and solute permeability measurements

Osmotically induced changes in vesicular volume are followed by measuring the change in scattered light intensity using a stopped-flow spectrophotometer as previously described [4]. One of the syringes of the stopped-flow apparatus is filled with a membrane suspension (0.1–0.3 mg protein/ml storage buffer as above) and the other syringe with the same solution with no membranes but, in addition, 300 mM mannitol, 300 mM urea or 150 mM NaCl to establish a hypertonic gradient upon mixing. Stopped-flow experiments were performed with an Aminco Morrow stopped-flow apparatus mounted on an Aminco DW-2a spectrophotometer. Scattered light intensity was measured under 90 degrees with the incident beam from the monochromator. The photomultiplier signal (preset at 700 V, high-voltage) was amplified 10-times with an Aminco photomultiplier microphotometer, bypassing the differential amplifier to eliminate signal noise from the chopper. The amplified signal was processed by a home-built data acquisition system and signals of five subsequent runs under identical conditions were recorded and summed using an Apple IIe. The summation without dividing increased the signal to noise ratio. The data were transferred to, and analyzed on, a Macintosh SE/30 computer, using a non-linear least-squares fitting algorithm, written in turbo pascal.

Immediately after applying a hypertonic gradient, water outflow occurs and the vesicles shrink. At the same time solute influx starts but the half-life of solute influx process is more than three orders of magnitude larger than the half-life of water outflow (see Results). Therefore, water outflow can be analyzed separately from solute influx. Osmotic shrinkage of the vesicles is fitted to either one or two exponential functions as before [4]. The solute influx or reswelling phase can also be fitted to either one or more exponential functions. The rate constants of the exponential functions are linearly related to permeability constants as previously demonstrated [4].

The data acquisition system can take in 2500 data points during one run. The frequency of sampling can be adjusted to obtain a good resolution in the first fast shrinking process as well as during the relatively slow solute influx process. The stopped-flow apparatus was thermostatically controlled. In experiments with the inhibitor, 30 min preincubation was needed with pCMBS while 15 min preincubation with $HgCl_2$ and 5 min preincubation with DMSO was sufficient. Preincubations were performed at room temperature. The medium osmolarity was measured by freezing point depression, using equipment from Precission Systems, Inc.

Results

The process of water outflow after mixing rat renal brush border membranes with a hypertonic mannitol solution is very rapid as shown in Fig. 1. Since the change in scattered light intensity is proportional to the change in vesicular volume [4] it can be concluded that 80 to 90% of the total shrinking process is completed within 50 ms for the upper control traces (0 mM DMSO) at the three experimental temperatures indicated. After the first fast shrinking process a second more slower shrinking phase is observed, of which the contribution to the total amplitude is only 10 to 20% in control experiments (0 mM DMSO).

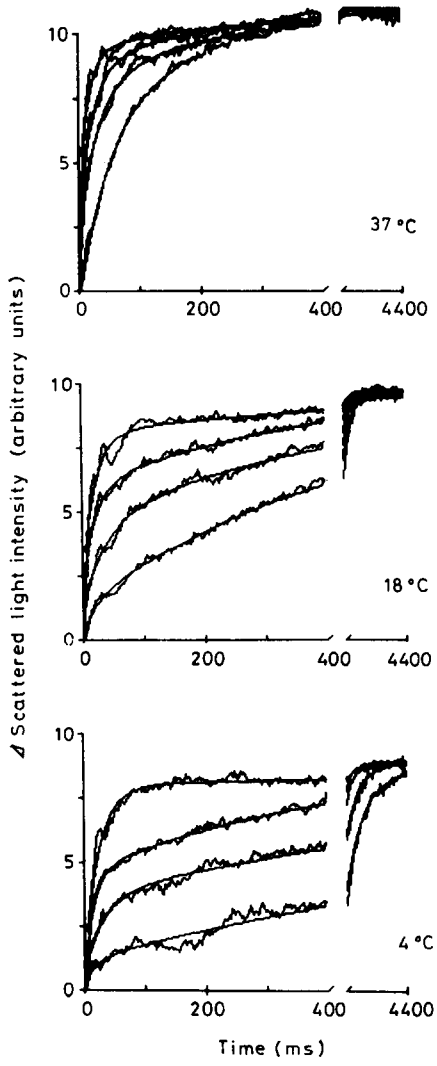

Fig. 1. Effect of dimethylsulfoxide on the time course of osmotic shrinkage of rat renal brush border membrane vesicles at three different temperatures. The sampling rate of the data acquisition system is decreased 10-fold after 400 ms and the increase in scattered light intensity is followed until completion (4.4 s). The vesicles are rapidly mixed with a hypertonic mannitol solution resulting in a 150 mM mannitol gradient (outside > inside). The protein concentration after mixing is approx. 0.125 mg/ml. The traces are summations of five single shots under identical conditions. A double exponential fit through the first 4.4 s is given for all traces. The four traces at each temperature show the effect of increasing DMSO concentrations (0, 100, 200 and 500 mM, from upper to lowest trace).

Two phenomena can be readily observed in the traces in Fig. 1. Firstly, addition of DMSO to the membrane suspension seems to decrease the amplitude of the first phase, apparently without much effect on the rate constant of the rapid phase. This peculiar effect of DMSO is more readily observed when experiments are performed at low temperature (Fig. 1). Secondly, the time constant of the second and slow phase seems more temperature-sensitive than the time constant of the first rapid phase. The inhibitory effect of DMSO is completely reversible, since washing the membranes restores the rapid phase of water outflow.

The effect of DMSO on the kinetics of osmotic shrinking of renal brush border membrane vesicles was studied in more detail by analyzing quantitatively the effect of a dose-response curve for DMSO. For comparison, the effect of DMSO on osmotic shrinkage of rat small intestinal brush borders was also studied. The result of this analysis is given in Fig. 2. Increasing concentrations of DMSO reduce the amplitude of the fast shrinking process which is presented as the percentage contribution of the fast component, ΔS_f, to the total amplitude of the osmotically-induced change in scattered light intensity, ΔS_t. In contrast to the decrease in amplitude, the rate constant of the fast shrinking phase is not altered significantly, for example in this particular experiment the k value ranges between 50 and 30 s^{-1}. The same holds for the rate constant of the second component of osmotic shrinkage. Although the amplitude of the slow component increases from 30 to 90% of the total signal amplitude, the rate constant only decreases from 2 to 1 s^{-1}. In intestinal brush border membrane vesicles the rapid phase of osmotic shrinkage is absent and DMSO has only a small effect on the time constant of the shrinking process (Fig. 2), comparable to the effect on the second component observed in osmotic shrinkage of renal vesicles.

The effect of $HgCl_2$ on osmotic shrinkage of renal brush border membrane vesicles is shown in Fig. 3. The effect of $HgCl_2$ is different when compared to the effect of DMSO, since $HgCl_2$ affects the rate constant but not the amplitude of the rapid osmotic shrinkage. Maximal inhibition of the fast shrinking phase is reached at 40 to

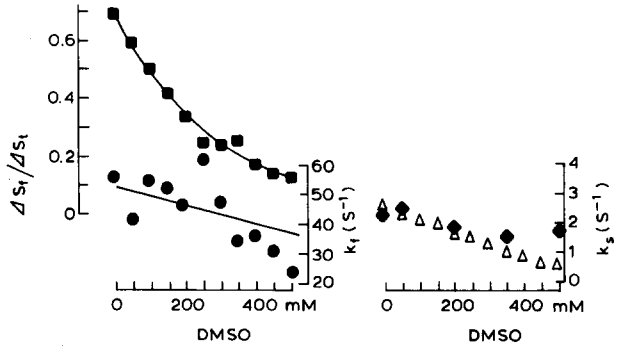

Fig. 2. Effect of dimethylsulfoxide on the fast osmotic shrinkage of renal brush border vesicles. The time course of osmotic shrinkage is measured as described in the legend of Fig. 1. Every concentration of DMSO is analyzed from summations of five single shots under identical conditions. The rate constants of the fast and slow processes are generated by computer from a double-exponential fit through the first 4.4 s. ●, k_f, rate constant of the fast process. △, k_s, rate constant of the slow process, ◆, rate constant of osmotic shrinkage of small intestinal brush border membrane vesicles. ■, $\Delta S_f/\Delta S_t$, amplitude of the fast shrinking process (S_f) divided by the amplitude of the total osmotic shrinkage (S_t). This particular set of experiments has been done at 4 °C and with an osmotic gradient of 150 mM mannitol.

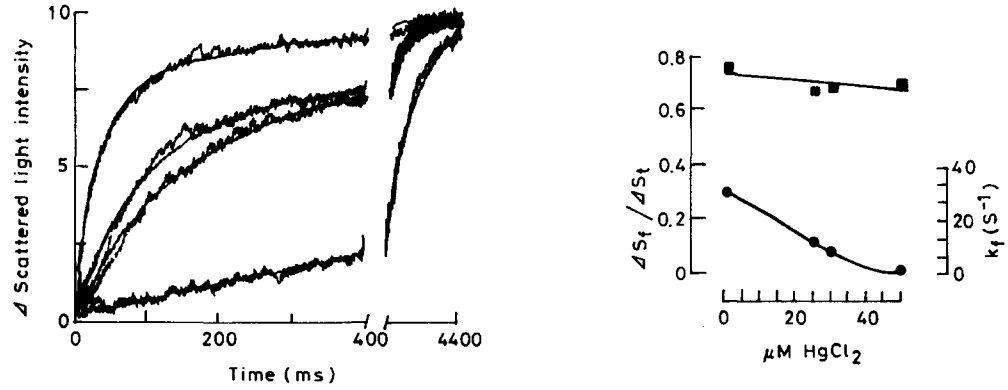

Fig. 3. Effect of $HgCl_2$ on the time course of osmotic shrinkage of rat renal brush border membrane vesicles. Osmotic shrinkage is induced by a gradient of 150 mM mannitol (o > i) at 4 °C. After 400 ms, the sampling rate is decreased 10-fold and the reaction was followed until 4.4 s. The traces are summations of five single shots under identical conditions and a double-exponential fit is shown. The four traces shown on the left represent experiments in the presence of 0, 25, 30 and 50 μM $HgCl_2$ (top to bottom). The panel on the right presents the rate constant of the fast shrinking process (k_f, ●) and the contribution of the fast component to the total change in amplitude ($\Delta S_f/\Delta S_t$, ■).

50 μM $HgCl_2$ with no effect on the second and slow component. This concentration also failed to affect the osmotic shrinkage of intestinal brush border vesicles (data not shown). To demonstrate more clearly the opposite effects of $HgCl_2$ and DMSO, we incubated renal brush border membranes in 200 mM DMSO and then studied the effect of increasing concentrations of $HgCl_2$ (Fig. 4A). In the second experiment we first incubated membranes in 25 μM $HgCl_2$ and then added increasing amounts of DMSO (Fig. 4B). In Fig. 4A, it is shown that 200 mM DMSO decreased the amplitude of the fast shrinking process to about 50% of the total amplitude. Addition of $HgCl_2$ now results in a decrease in the rate constant k_f but not in amplitude. At $HgCl_2$ concentrations with maximal effect there is only one slow shrinking process left ($\Delta S_f/\Delta S_t$ presented as 1 in Fig. 4A). The opposite experiment in Fig. 4B demonstrates that 25 μM $HgCl_2$ has decreased the rate constant of the first phase to around 30 s^{-1}. Addition of DMSO now results in a decrease in amplitude without changing the time constant of the fast component.

The effects of DMSO and $HgCl_2$ on the apparent activation energy of osmotic water flow is shown in Fig. 5. The upper trace gives the temperature dependence of

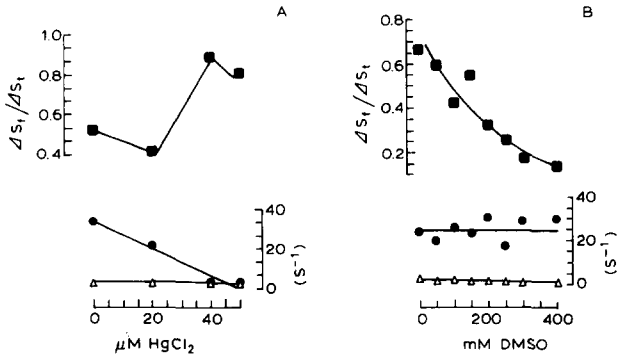

Fig. 4. Simultaneous effects of $HgCl_2$ and dimethylsulfoxide on the time course of osmotic shrinkage of rat renal brush border membrane vesicles. Panel A: membranes are preincubated with 200 mM DMSO and subsequently treated with 0, 20, 40 and 50 μM $HgCl_2$. Panel B: membranes are preincubated with 25 μM $HgCl_2$ and subsequently treated with increasing concentrations of DMSO. ■, $\Delta S_f/\Delta S_t$, change in amplitude of the fast component divided by the change in amplitude of the total scattered light signal. ●, k_f, rate constant of the fast shrinking phase. △, k_s, rate constant of the slow shrinking phase. The rate constants are generated by computer from a double-exponential fit through the first 4.4 s. Traces are summations of five single shots under identical conditions (temperature 4 °C, osmotic gradient 150 mM mannitol).

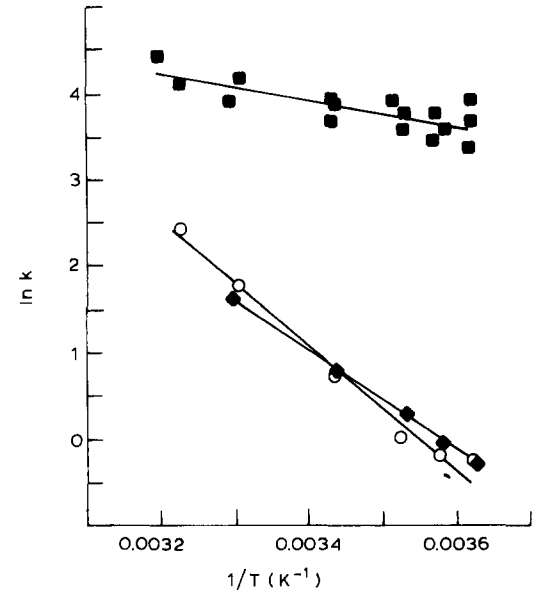

Fig. 5. The temperature dependence of the rate constant of osmotic shrinkage of rat renal brush border vesicles. ■, k_f, rate constant of fast shrinking process. ◆, k, rate constant of osmotic shrinkage in the presence of 50 μM $HgCl_2$. ○, k, rate constant of osmotic shrinkage in the presence of 500 mM DMSO. Osmotic shrinkage is induced by a 150 mM mannitol gradient (o > i). Rate constants are generated by computer from a double-exponential fit (control) and a single-exponential fit (in the presence of $HgCl_2$ or DMSO) through the traces. Traces are summations of five single shots under identical conditions.

TABLE I

Osmotic water permeability of renal brush border membrane vesicles

P_f values are calculated from: $k = \bar{V}_w \cdot P_f \cdot (A/V_0) \cdot (1-(b/V_0))^{-1} \cdot \Delta C$ (Ref. 4) in which k = rate constant of osmotic shrinkage, $\bar{V}_w$ = molar volume of water, P_f = osmotic water permeability, A/V_0 = surface of vesicles/initial volume of vesicles ($V_o/A = 35$ nm) and $(1-(b/V_0))$ osmotic active volume of vesicle = 0.72 (Ref. 4), ΔC = osmotic gradient.

	Osmotic water permeability (cm/s)		
	P_f (fast) control	P_f (500 mM DMSO)	P_f (50 μM $HgCl_2$)
37 °C	0.076	0.011	0.008
3 °C	0.039	0.0007	0.0008
Activation energy (kcal/mol)	3.1	14.5	11.5

the rate constant of the fast osmotic shrinkage. The lower traces represent the rate constant of osmotic shrinkage after maximal inhibition of the fast shrinking process. In Table I, the actual values for the osmotic water permeability and for the activation energy are given, calculated from the data in Fig. 5. After treatment with DMSO or $HgCl_2$, the activation energy for water permeation increases to values typical for diffusion through a lipid environment.

In the literature, *p*-chloromercuribenzenesulfonic acid (pCMBS) is often used to inhibit water movement through channels [1–3]. Fig. 6 shows the effect of 10 mM pCMBS on osmotic shrinkage. The trace recorded at 10 °C is given as a typical example, but the effect was studied between 37 and 3 °C as in the studies with

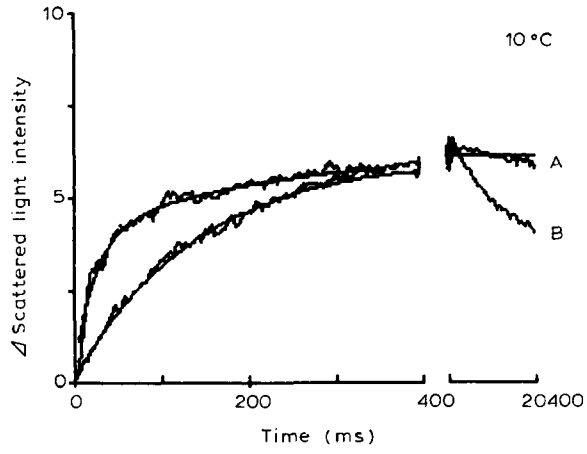

Fig. 6. Effect of *p*-chloromercuribenzenesulfonic acid on the time course of osmotic shrinkage of rat renal brush border membrane vesicles. Osmotic shrinkage is induced by a 150 mM mannitol gradient. The sampling rate is decreased 50-fold after 400 ms in order to follow the signal up to 20.4 s. Trace A, untreated control. Trace B is obtained in the presence of 10 mM pCMBS (30 min preincubation at room temperature). Both traces are summations of five single shots under identical conditions. A double-exponential fit is shown through the first 400 ms.

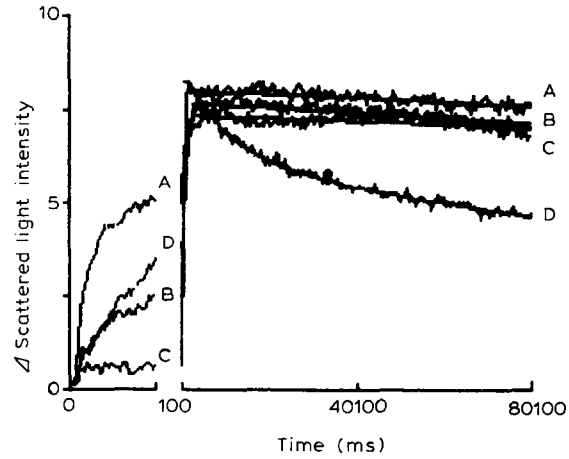

Fig. 7. Effects of DMSO, $HgCl_2$ and pCMBS on the time course of osmotic shrinkage and reswelling of rat renal brush border membranes. Osmotic shrinkage is induced by a 150 mM mannitol gradient. Traces are summations of five single shots under identical conditions at 4 °C. The sampling rate is decreased 200-fold after 400 ms in order to follow the reswelling-phase up to 80 s. A single exponential fit to the reswelling-phase is shown. Trace A: control, B: 40 μM $HgCl_2$ present, C: 500 mM DMSO present, D: 10 mM pCMBS present.

DMSO and $HgCl_2$. The results with pCMBS resemble those found with 50 μM $HgCl_2$ on the rapid water outflow process at temperatures between 37 and 20 °C. Below 20 °C, pCMBS was less effective than $HgCl_2$. In addition, pCMBS markedly increased the rate constant of reswelling of the vesicles, indicating that the influx of mannitol was accelerated. In two experiments we com-

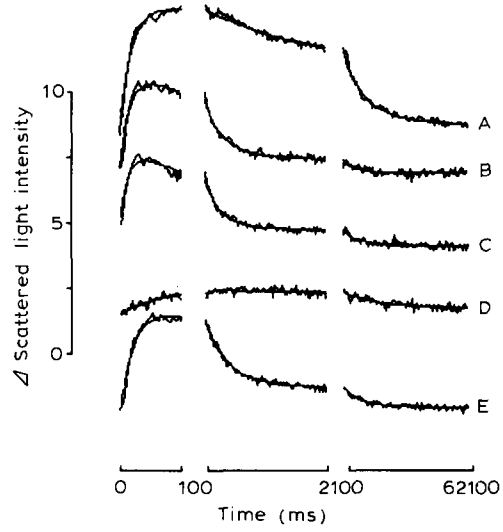

Fig. 8. Effect of $HgCl_2$ on urea permeability of rat renal brush border membrane vesicles. Osmotic shrinkage is induced by a 150 mM urea gradient (o > i). The sampling rate is decreased 10-fold after 100 ms and again 30-fold after 2.1 s in order to follow the reswelling-phase untill completion. Traces are summations of five single shots under identical conditions at 10 °C. Trace A: control, B: 10 μM $HgCl_2$ present, C: 10 μM $HgCl_2$ preincubation followed by 1 mM dithiothreitol, D: 100 μM $HgCl_2$ present, E: 100 μM $HgCl_2$ preincubation followed by 1 mM dithiothreitol. Exponential fits to the shrinkage and reswelling-phases are shown.

TABLE II

Effects of $HgCl_2$ on water, NaCl and urea permeabilities of rate renal brush border permeabilities

Rate constants k_f for fast osmotic shrinkage and k for solute influx are generated by computer from exponential fits through the traces A to E as shown in Fig. 8. Mean values of two experiments at 10 °C are given. n.a., not analyzed since the amplitude of the osmotic response was too small. Osmotic gradients: 150 mM urea, 75 mM NaCl.

Conditions	Rapid shrinkage (water outflow)		Reswelling (solute influx)	
	NaCl k_f (s^{-1})	urea k_f (s^{-1})	NaCl k (s^{-1})	urea k(s^{-1})
a. Control	49.0	57.0	0.05	0.16
b. 10 μM $HgCl_2$	52.0	58.0	0.86	1.9
c. 10 μM $HgCl_2$ +1 mM DTT	53.0	62.0	0.79	1.8
d. 100 μM $HgCl_2$	3.6	3.1	n.a.	n.a.
e. 100 μM $HgCl_2$ +1 mM DTT	45.0	40.0	0.69	2.0

pared the effects of DMSO, $HgCl_2$ and pCMBS on reswelling of renal brush border vesicles in mannitol solutions. In both experiments, neither 500 mM DMSO nor 40 μM $HgCl_2$ could change mannitol permeability (rate constant for mannitol influx around $6 \cdot 10^{-4}$ s^{-1}). Only 10 mM pCMBS increased the rate constant for mannitol influx to $35 \cdot 10^{-4}$ s^{-1} (Fig. 7).

Using NaCl or urea instead of mannitol as the osmotic agent, it could be shown that $HgCl_2$ at low concentrations dramatically increased the rate constant of solute influx. In Fig. 8, a series of experiments in which the effect of $HgCl_2$ on urea influx into renal brush border membrane vesicles is shown. In Table II,

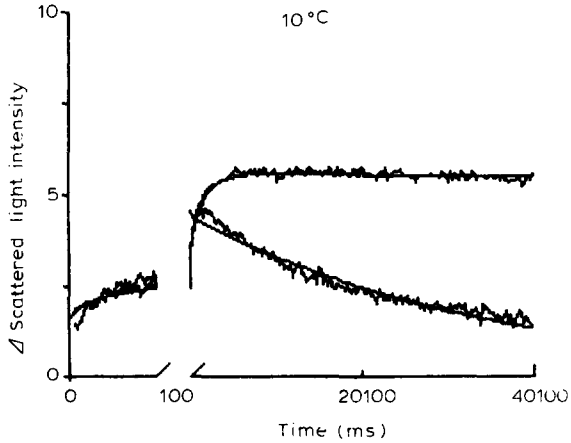

Fig. 9. Time course of osmotic shrinkage and reswelling of phospholipid vesicles induced by NaCl (upper trace) and urea (lower trace) gradients. Osmotic shrinkage is induced by a 150 mM urea or a 75 mM NaCl gradient. Traces are summations of four single shots at 10 °C. Exponential fits are shown through the shrinkage and reswelling-phase. The rate of sampling is decreased 100-fold after 100 ms. The vesicle density after rapid mixing was 0.5 mg asolectin/ml.

TABLE III

Effects of DMSO, $HgCl_2$ and pCMBS on water NaCl and urea permeabilities of asolectin vesicles

Rate constants (in s^{-1}) are generated by computer from exponential fits through traces as shown in Fig. 9. Mean values of two experiments are given.

	k_{water}	k_{urea}	k_{NaCl}
Control	1.8	$2.8 \cdot 10^{-2}$	$8.9 \cdot 10^{-7}$
500 mM DMSO	2.1	$2.7 \cdot 10^{-2}$	$8.0 \cdot 10^{-7}$
40 μM $HgCl_2$	2.3	$3.2 \cdot 10^{-2}$	$8.8 \cdot 10^{-7}$
10 mM pCMBS	1.9	$2.8 \cdot 10^{-2}$	$9.0 \cdot 10^{-7}$

the rate constants are given for osmotic shrinkage and reswelling induced by NaCl and urea. It is clear that 10 μM $HgCl_2$ increases urea and NaCl permeability by more than one order of magnitude while water permeability is not yet affected. At higher $HgCl_2$ concentrations the rate of water outflow is also decreased. A striking observation is the reversibility of the inhibition of water flow by dithiothreitol compared to the irreversibility of the effect on solute influx. DMSO had no effect on the permeability for NaCl and urea, since the k values for both solutes were 0.04 and 0.14 s^{-1}, respectively, in the presence of 500 mmol at 10 °C (see Table II for control values).

The effects of DMSO, $HgCl_2$ and pCMBS were also studied on the permeability of pure phospholipid vesicles to investigate whether the inhibitors used affect the lipid structure. Fig. 9 shows the increase in scattered light intensity when asolectin vesicles are subjected to a hypertonic osmotic shock of 150 mM urea or 75 mM NaCl. The osmotic shrinking process is much slower than that of renal brush border membrane vesicles. The phospholipid vesicles also proved to be rather impermeable to NaCl since, at the time scale indicated, no reswelling due to NaCl influx could be observed. The presence of 500 mM DMSO, 500 μM $HgCl_2$ or 10 mM pCMBS did not influence the traces in Fig. 9 (see Table III), indicating that these compounds do not influence water and solute permeability of pure phospholipid membranes.

Discussion

The interaction of DMSO, $HgCl_2$ and pCMBS with water and solute permeability of brush border membrane vesicles from rat renal cortex was studied by measuring the kinetics of changes in scattered light intensity upon rapid osmotic perturbation using stopped-flow spectrophotometry. Osmotic water transport in these membranes has been reported previously to be functionally heterogeneous, consisting of two distinct vesicle populations with different water transport properties [4,13,23]. Two distinct rates of osmotic water

flow were again observed in the present study. At low temperatures, the two rates of osmotic shrinkage are distinguished much more easily than at 37°C, because the fast component is less temperature-sensitive than the slow component. The second and slow component reflects water permeation through a lipid environment and has a higher activation energy than water permeation through a water environment (Fig. 5). At 5°C, the rate constants of the fast and slow processes are almost two orders of magnitude apart (Table I). Since there are no break points in the Arrhenius plots given in Fig. 5, we conclude that the mechanism of water permeation is not changed by lowering the temperature from 37 to 5°C. For this reason, most of the experiments in this study have been performed at 5°C to increase the reliability of fitting the observed traces to two exponential functions.

In the present study around 80% of the osmotic shrinkage of renal membrane vesicles is fast, compared to 50% in a previous study [4]. This difference was introduced by the fact that in the present study, 3–5-month-old rats were used instead of 2-month-old ones previously. The biexponential kinetics of osmotic shrinkage can only be explained by a heterogeneity in the vesicle preparation, i.e., 80% containing water channels and 20% contamination with membranes lacking such structures. This heterogeneity is typical for renal cortical plasma membrane preparations and is not observed in plasma membrane vesicles from small intestine or colon, membranes which lack water channels [4,19].

We report for the first time that DMSO blocks water movement through channels in renal membranes. Since DMSO decreased the water permeability without affecting the rate constant of water movement, DMSO decreases the number of vesicles contributing to the fast shrinking process. This means that DMSO closes all the channels in some vesicles, the number of which increases in a dose-dependent manner.

From the effects of $HgCl_2$ on osmotic water permeability of renal membranes, we postulate a different mechanism of inhibition by mercurials. $HgCl_2$ decreased the rate constant without lowering the contribution of the fast component to the total change. This implies that $HgCl_2$ does not close all the channels in some vesicles but rather some channels in all the vesicles and thus decreases the rate constant of osmotic shrinkage by reducing the mean osmotic water permeability of the vesicles. In other words DMSO may act in an 'all-or-nothing' fashion by blocking all channels in one vesicle, while $HgCl_2$ seems to close one channel in all the vesicles in an 'all-or-nothing' fashion. The absence of effects of DMSO and $HgCl_2$ on the water permeability of small intestinal brush border membrane vesicles and asolectin vesicles strongly supports the view that the target of these inhibitors of water permeability in renal brush border membranes is a specific protein rather than the lipid matrix.

Since DMSO is added in rather high concentrations alternative explanations for its effects have to be discussed. A decrease in amplitude of the fast component could also result from a reduced intravesicular to extravesicular osmotic gradient. To test whether DMSO interfered with applied osmotic gradients we followed vesicular volume upon application of a DMSO concentration gradient. In several attempts we were unable to observe vesicular shrinkage after rapid mixing with DMSO containing solutions. Therefore we conclude that the reflection coefficient of DMSO is not different from 0 and DMSO is not capable of modifying the applied mannitol gradients.

$HgCl_2$ and DMSO increased the apparent activation energy of water permeation from values typical for water movement through a water environment to values typical for water movement through a lipid environment (Fig. 5). This result strongly supports the conclusion that both agents close the water channels in the membrane, leaving the diffusive pathway through the lipid matrix as the only mechanism for water permeation.

In the studies with intact renal tubules or vasopressin-responsive amphibian epithelia, there seems to be a preference for using pCMBS as the water channel inhibitor [7,20,21]. In our vesicle study, pCMBS was not as effective as $HgCl_2$, especially at lower temperatures. In addition, pCMBS has to be used in rather high concentrations which, in our study, induced non-specific solute leaks; for example, pCMBS induced an increase in mannitol permeability while $HgCl_2$ did not.

The sensitivity of the water channel in proximal tubule brush border membranes for $HgCl_2$ found in the present study is comparable to the $HgCl_2$ sensitivity reported for the red cell water permeability ($K_D \approx 0.02$ mM) [3]. The water permeability in vasopressin-induced endocytic vesicles from toad urinary bladder was far less sensitive for $HgCl_2$ [22]. The lower inhibitory potency of $HgCl_2$ in toad bladder endocytic vesicles compared with proximal tubule vesicles suggests that the vasopressin-responsive water channel is different from the water channel in proximal tubules. Despite this difference in inhibitory potency of $HgCl_2$, inhibition of the water channel by $HgCl_2$ was reversible in both preparations [22].

When NaCl or urea was used instead of mannitol, we observed that $HgCl_2$ at very low concentrations increased the permeability for NaCl and urea 15-fold. This increase in solute leak preceded the inhibition of water permeability. Since the increase in leakage of small solutes could not be reversed by cysteine or dithiothreitol, we suggest that, at low concentrations, $HgCl_2$ destroys the tertiary structure of membrane-spanning proteins, other than those are responsible for

the high water permeabilities. An observation in support of this suggestion is that $HgCl_2$ had no effect on NaCl and urea permeability of asolectin vesicles.

The dual effect of $HgCl_2$ on renal brush border membrane vesicles observed in the present study implies a complete dissociation of solute and water pathways. This should be reflected in the reflection coefficients for NaCl and urea since the reflection coefficient (σ) of a solute is an index to the extent of solute and water interaction in the membrane [23]. σ is generally determined by comparison of water flows induced by different solutes for a given osmotic gradient [23]. Mannitol, as a low permeability molecule for which $\sigma = 1$, can be used as reference. The reflection coefficient for NaCl is than equal to:

$$\sigma = J_v(\text{NaCl})/J_v(\text{mannitol}) = k_f(\text{NaCl})/k_f(\text{mannitol})$$

in which J_v(NaCl) is the volume flow induced by NaCl and k_f(NaCl) is the rate constant of the osmotic shrinkage induced by NaCl [24]. Throughout the study we found k_f(mannitol) values of approx. 50 s^{-1} (Figs. 2, 3). Similar k_f(NaCl) or k_f(urea) values are listed in Table II. Therefore, reflection coefficients for urea and NaCl are approx. 1, implying a lack of interaction between urea or NaCl and water. Even when 10 μM $HgCl_2$ has increased NaCl and urea permeability by more than one order of magnitude, the reflection coefficient for urea and NaCl remains approx. 1 (Table II, row b). Only after inhibition of the water permeability by one order of magnitude with 100 μM $HgCl_2$, are the reflection coefficients for urea and NaCl decreased to values below 0.1 (Table II, row d), bearing in mind that $HgCl_2$ does not change the mannitol permeability. By increasing the rate of solute influx 15-fold and by decreasing the rate of water outflow 10-fold, the shrinkage and reswelling-processes are no longer separated in time and the amplitude of the change in scattered light intensity decreases (trace d in Fig. 8).

In conclusion, water and solute pathways in rat renal brush border membrane vesicles are completely separate entities, which are affected differently by mercurial sulfhydryl reagents and which must be proteic in nature.

Acknowledgements

This work was supported by a grant from The Netherlands Organization for the advancement of Pure Research (NWO) under the auspices of The Netherlands Foundation for Biophysics. We are indebted to Dr. J.A. Dempster for critical reading and to Mrs. S.E. Engels for editorial assistance.

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