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EFFECT OF *para*-CHLOROMERCURIBENZENESULFONIC ACID AND TEMPERATURE ON CELL WATER OSMOTIC PERMEABILITY OF PROXIMAL STRAIGHT TUBULES

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The apparent Arrhenius energy of activation (E_a) of the water osmotic permeabiity (P_{os}^c) of the basolateral plasma cell membrane of isolated rabbit proximal straight tubules has been measured under control conditions and after addition of 2.5 mM of the sulfhydryl reagent, *para*-chloromercuribenzenesulfonic acid (pCMBS), of mersalyl and of dithiothreitol. E_a (kcal/mol) was 3.2 ± 1.4 (controls) and 9.2 ± 2.2 (pCMBS), while P_{os}^c decreased with pCMBS to 0.26 ± 0.17 of its control value. Mersalyl also decreased P_{os}^c both in vitro and in vivo (using therapeutical doses). These actions of pCMBS and mersalyl were quickly reverted with 5 mM dithiothreitol and prevented by 0.1 M thiourea. E_a for free viscous flow is 4.2 and greater than 10 for non-pore-containing lipid membranes. By analogy with these membranes and with red blood cells, where similar effects of pCMBS on P_{os} are observed, it is concluded that cell membranes of the proximal tubule are pierced by aqueous pores which are reversibly shut by pCMBS. Part of the action of mercurial diuretics can be explained by their action on P_{os}^c.

Introduction

The transepithelial water movements observed during secretion or absorption in leaky epithelia, particularly across the mammalian proximal tubule, are envisaged to follow a transcellular and/or a paracellular pathway [1–5]. This paper is not concerned with the paracellular route for water flow but rather with water movements between cells and peritubular space and, therefore, with transcellular water movement. Although there is general agreement in that a significant fraction of the transepithelial water movement is transcellular [5–11], the mechanism used by water to cross the cell membrane has not been studied in leaky epithelia. There exist two types of possibilities. Water may be assumed to be dispersed in the lipid-rich cell membrane and/or aggregated in the cell membrane such that it forms more or less continuous files or 'pores' stretching across the cell membrane from extra to intracellular space, i.e., from one aqueous phase to another [12]. In agreement with these possibilities, the study of mammalian red blood cells and lipid artificial membranes had led to the conclusion that water flows in two ways across these membranes. (i) In the first place, as is observed in artificial lipid membranes (without pores) water dissolves in the membrane and crosses it strongly interacting with the membrane fabric, as evidenced by very low values for the water osmotic permeability P_{os} (which are of the order of the permeability for water diffusion (P_d) and by apparent Arrhenius energies of activation (E_a) [12–15,22] of the order or higher than 12–14

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Abbreviation: pCMBS, *para*-chloromercuribenzenesulfonic acid.

kcal/mol (the exact value depending on the lipid composition of the membranes) [12–15]. (ii) In addition, water may cross the membranes by viscous flow. This type of flow is observed by introduction of some antibiotics into the lipid bilayers. There is evidence that these antibiotics form across the lipid membrane small hydrophilic channels (0.2–0.4 mm in radius) that can accomodate ions and water molecules [12]. As compared with (i), the presence of even small aqueous pores increases the water flux for a given water-activity gradient because they permit the transfer of bulk momentum between water molecules [12,14–20]. P_{os} is without doubt higher than P_d in pore-containing artificial membranes [12]. In addition, E_a of P_{os} is close to that expected for free solution viscous flow (i.e., 4.2 kcal/mol) as is the case of mammalian red blood cells where P_{os} is also significantly larger than P_d [16–22]. Addition of pCMBS, reversibly lowers P_{os} in red blood cells to values about 0.1 of those obtained under control conditions. Under these circumstances P_{os} approaches the value of P_d, and E_a of pCMBS-treated cells increases to values observed in nonporous lipid membranes. Thus, pCMBS seems to interact with the membrane, reversibly closing the water-porous pathways normally present under control conditions in red blood cells [16–18,20].

In tight epithelia, there is still controversy concerning the existence of pores after antidiuretic hormone stimulation. Ambiguity arises from uncertainties of unstirred layer effects and other factors [9,23]. However, new independent measurements of the diffusion permeabilities to water and to molecules with various lipid solubilities have provided evidence indicating that water probably crosses the luminal cell membrane primarily through small aqueous pores even in the unstimulated state, antidiuretic hormone stimulation leading either to formation or enlargement of pores [23]. Concerning leaky epithelia, technical difficulties still preclude measurement of permeability to diffusion. It has only been recently that satisfactory measurements of the water osmotic permeability have been reported in amphibian gall bladder [11] and of P_{os}^c, the basolateral cell membrane water osmotic permeability in mammalian isolated tubules [6–10,24]. The relative shortcomings of the techniques employed to measure the time course of cell swelling (to estimate P_{os}^c) and therefore the accuracy of these measurements [6,8,10] have been pointed out [7], indicating either insufficient space resolution with excellent time measurements [10] or excellent space resolution with less precise time controls [6,8].

Although, in general, aqueous channels are presumed to cross the cell membrane of these tissues [7], evidence as clear cut as that reported for red blood cells and for artificial membranes is still missing. To gain some insight into the nature of the water movements across kidney proximal tubular cells, we investigated the effect of temperature and of sulfhydryl reagents on P_{os}^c in mammalian isolated tubules. A new technique with good space and time resolution has been used to measure P_{os}^c [24]. pCMBS was chosen because of its specific action on water transfer over that of other reagents in red blood cells [25]. We have studied: (i) The effect on P_{os}^c of pCMBS and of dithiothreitol, an agent which is used for the quantitative reduction of disulfide groups and which reverses the action of pCMBS in red blood cells [16–19]. (ii) The E_a of control and of pCMBS-treated tubules. (iii) Similar experiments were performed using the mercurial diuretic mersalyl, instead of pCMBS. The present experimental results indicate that E_a of control experiments is of the order of that expected for viscous flow. pCMBS significantly reduces P_{os}^c and increases E_a. Mersalyl has a similar action. It is possible that part of the diuretic action of this compound might be related to the decrease in the P_{os}^c it produces.

Materials and Methods

The method used to handle the tubules and to measure P_o^c has been described in detail before (Ref. 24, see also Refs. 6, 8). New Zealand rabbits weighing 1.5–2 kg were used. Individual proximal straight tubular segments (about 0.5 mm in length) were dissected out of the upper kidney cortex. They were crimped with micropipettes in a chamber leaving a length of 0.2–0.3 mm of usable free tubular tissue between the pipettes, and then were kept in an isosmotic artificial bathing solution which was equilibrated with 95% O_2/5% CO_2 previously thermostated at the desired temperature. The chamber was thermostated with the aid of a

surrounding water jacket. The inverted microscope was focused on the tubular axis using a 40× objective. The tubular image was recorded with a TV camera. The video signal was analyzed with a special processor which generates an analog signal, proportional to the outer tubular diameter, to a pen-recorder. Following the records, tubular volume was calculated as a function of time. The time resolution was 0.0167 s and the space resolution was near 0.03 μm [24]. For the osmotic steps required to measure P_{os}^{c}, the chamber was quickly emptied and the anisosmotic test medium was 95% replaced about the tubule in less than 100 ms [6]. When the diameter change was stabilized, the isosmotic bathing solution was placed back in the chamber. Some of the experimental protocols required exposition of the tubules in the chamber to different solutions for times as long as 1–2 h. At 37 °C, this might have damaged the tubules. Therefore, they were kept crimped in the chamber at 10–15 °C, except for the short periods of time in which P_{os}^{c} was measured at the required temperature.

Bathing solutions. All solutions were made up from a basic solution containing (in mM) KCl, 4.8/$NaHCO_3$, 25.0/$MgSO_4$, 1.2/$CaCl_2$, 1.9/Na_2HPO_4, 4.0/glucose, 8.3/alanine, 5.0. Bovine serum albumin (6 g/dl) was added to this solution. Required amounts of NaCl were added to the basic solution to achieve an osmolality of 300 mosM. This solution was used to equilibrate the tubules. Dextran M_r 60000 (1 mM), was used instead of albumin to prepare the pCMBS-containing solutions to avoid reaction between this agent and the disulfide bonds of albumin [26,27]. Osmolality differences across the peritubular aspect of the cells were achieved by using solutions with different NaCl concentrations. The osmolality of the solution was checked by freezing-point determination. All chemicals were obtained from Sigma Chemical Co. Ltd.

Calculation of P_{os}^{c}. This has been described in detail [6,8,24]. The tubular lumen was collapsed, thus only water flow through the contraluminal (basolateral) membrane could contribute to the volume change. Briefly, water flow from cell to bath or bath to cell is provoked by a step change of the osmotic pressure difference across the cell membrane, $\Delta C_o^i = C_c - C_{out}$. This is achieved by increasing or decreasing C_{out}, the concentration of impermeant solutes in the bath. C_c is the cellular concentration of osmotically active substances. Cell volume changes are followed as diameter changes [6,8,24]. Cell volume, V^c, and C_c will change with time. Therefore, the osmotic pressure difference across the cell membrane will also change with time. P_{os}^{c} may be obtained from Eqn. 1

$$P_{os}^{c} = \left[V_{iso}^{c}/(A_{iso}^{c} C_{out} t)\right]\left[1 - R + (b - K) \times \ln\left|(K - R)/(K - 1)\right|\right] \quad (1)$$

where A_{iso}^{c} is the membrane area, $R = V^c/V_{iso}^{c}$, the ratio of tubule volume at times t and 0, respectively, and $K = (C_{iso}^{c}/C_{out})(1 - b) + b$, where C_{iso}/C_{out} is the ratio of external concentration before and after the solution change. Before the osmotic experiment begins, $C_c = C_{iso}$, since the cells have an intracellular concentration isosmotic to that of the bath in which the tubule is kept. b is the cell solid content. Eqn. 1 was fitted to the data to calculate P_{os}^{c}.

Results

Most experiments were carried out with an osmotic difference of 30 mosM. Under the conditions at 37 °C, the control values for P_{os}^{c} were $(21.3 \pm 4.3) \cdot 10^{-4}$ cm³/s per osM per cm² of basal membrane ($n = 12$). This value agrees with previous estimates at the stated osmotic difference [24] and corresponds to an extrapolated value (to 0 osmotic difference) of $50.4 \pm 8.7 \cdot 10^{-4}$ in the same units [24]. This value equals $18.7 \cdot 10^{-8}$ cm³/s per cm H_2O per cm² of basement area, $19.2 \cdot 10^{-5}$ cm³/s per atm per cm² of basement membrane area and 2800 μm/s and agrees with independent measurements [10]. In what follows, results are expressed as ratios of experimental to control values.

Effect of pCMBS on P_{os}^{c}

To test the effect of pCMBS, we proceeded as follows. First a series of five control measurements were performed at 37 °C with a given tubule at 1-min intervals, each measurement taking some 10 s. Following each return to isosmotic solution, the tubule did return to its original diameter. The

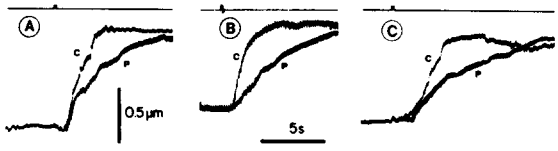

Fig. 1. Actual records of the time course of diameter (volume) changes, Pannels A, B and C represent experiments with three different tubules. Two records are shown in each pannel. In records C (control condition), tubules had been equilibrated in an isosmotic (0.3 osM) solution. At a time indicated by a notch in the top horizontal line, a hyposmotic solution (0.27 osM) was flushed in and diameter changes were recorded. The slope of the diameter changes as function of time was used to obtain P_{os}^{c}. Subsequently, an isosmotic solution was flushed in. An isosmotic solution containing 2.5 mM pCMBS was replaced as the bathing solution for 25 min. Then record P was obtained with the same osmotic difference. The time notch was used to superimpose both traces.

control values agreed within 3%. Then, the tubule was incubated for 25 min at 10–15°C in the presence of a solution containing 2.5 mM pCMBS, and P_{os}^{c} measurements were performed again at 37°C. Some of the actual records are reproduced in fig. 1. It may be seen that pCMBS diminishes the speed of the osmotic response and the value of P_{os}^{c} (as compared to the controls) without altering the end-point of the osmotic response.

The values for P_{os}^{c} could be falsely low if the membrane permeability to Na were to increase after pCMBS treatment so that the reflection coefficient for Na could be near 0 rather than closer to 1 as in the control condition [24]. Measurements of the reflection coefficient (not reported here) indicate that this is not the case. There was no difference in the osmotic response to osmotic steps induced with NaCl and with mannitol or raffinose to measure the reflection coefficient. This indicates that the reflection coefficient for NaCl, mannitol and raffinose is 1.0.

Although we do not know whether pCMBS altered the cell composition (as is the case of red cells [28] and frog skin [29]), the possibility that the initial osmotic step across the membrane would be lower than that obtained under control conditions can also be ruled out, since the tubules in the presence of pCMBS responded osmometrically as illustrated by the observation that control and pCMBS records reach the same end-point. In this work, we preferred NaCl to the non-electrolytes as osmotic agents since the latter change the viscosity of the solution which is undesirable if E_a is to be evaluated. In addition, the diffusion coefficient of these non-electrolytes is a fraction of that for NaCl. This shows up in unstirred layer effects [6,8].

Fig. 2 shows a dose-response curve. It may be seen that the concentration of pCMBS required to produce half-maximal effect, $K_{0.5}$, is near 0.12 mM. This value agrees with that observed in red cells [14]. In the experiments which follow, a concentration of 2.5 mM pCMBS was used in order to obtain maximal and clear-cut differences between controls and pCMBS-treated tubules in a relatively short time (25 min).

As seen in Fig. 3, 2.5 mM pCMBS lowers P_{os}^{c} to about 0.5 in about 15 min and to about 0.2 in 20–30 min. This time course is similar to that observed in red cells [16,17]. The time required for the chemical reaction of mercurials with proteins in aqueous solution ranges from minutes to hours [27]. In erythrocytes, times of incubation of 20–30 min need be used [16,17] and the uptake of pCMBS over a longer time period at 37°C could produce a similar action to that of 2.5 mM pCMBS used here, as in the case of mersalyl (Fig. 3). However, the experimental protocol called for measurements under control conditions followed by pCMBS and subsequent addition of dithiothreitol to check on reversal of the action of pCMBS. This rendered longer pCMBS exposure times impractical. It may

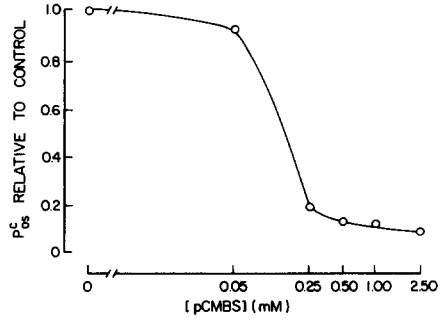

Fig. 2. Dose-response curve showing the action of pCMBS on P_{os}^{c}. P_{os}^{c} is expressed as a fraction of its control value. In each experiment after five control measurements of P_{os}^{c}, the tubules were incubated with pCMBS for 25 min, and P_{os}^{c} was measured again. The graph shows average results obtained in five tubules. The osmotic difference used was 30 mosM. The temperature was 10°C throughout.

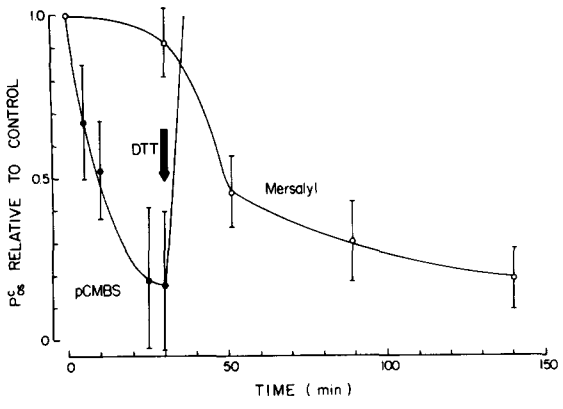

Fig. 3. Time course of the action of 2.5 mM pCMBS (●) and of 100 μM mersalyl (○) on P_{os}^c. Values in the presence of the drugs ($n = 12$) are expressed as relative to a set of controls ($n = 12$) run in parallel. At the time indicated by the arrow, 5 mM dithiothreitol (DTT) was added to the pCMBS-containing solution.

also be seen in Fig. 3 that the action of pCMBS could be reverted after a short exposition to 5 mM dithiothreitol. We did not explore whether lower concentrations of dithiothreitol also reverted the action of pCMBS or whether lower dithiothreitol concentrations had a different reversal time course. Dithiothreitol alone does not significantly alter P_{os}^c. Thus, in a series of 12 experiments, the ratio of P_{os}^c values of dithiothreitol-treated to control tubules (the same tubule was used as its own control previous to the addition of dithiothreitol) was 1.30 ± 0.16 ($n = 12$) (mean ± S.E.), a value not statistically different from 1.00. In a separate series of 12 experiments, values for P_{os}^c were measured under control conditions. Then, pCMBS was added and P_{os}^c was measured after 25 min. Use of 2.5 mM pCMBS for 25 min decreased P_{os}^c to 0.26 ± 0.17, a figure statistically different from 1.00 ($t = 4.35$, $p < 0.001$) but not from 0. After the measurement in the presence of pCMBS, 5 mM dithiothreitol was added and P_{os}^c was measured again. After 10 min, P_{os}^c was 1.91 ± 0.26, a value significantly higher than 0.26 ± 0.17, but not statistically different from 1.30 ± 0.16 obtained in the group of tubules treated with dithiothreitol alone.

Prevention of pCMBS action by thiourea

As is the case of erythrocytes, 0.1 M thiourea prevented the action of 2.5 mM pCMBS [19]. This series of experiments were performed as follows. First, control measurements of P_{os}^c were performed. Then, the tubules were bathed in a solution containing thiourea for 5 min and P_{os}^c was again measured. The ratio of thiourea/control value was 1.51 ± 0.21 ($n = 12$). Subsequently, and still in the presence of thiourea, 2.5 mM pCMBS was added and after 30 min, a group of P_{os}^c measurements was performed again. The average value of P_{os}^c as referred to control values was 1.62 ± 0.31 ($n = 12$) which is not different from the average ratio of 1.51 obtained previously. As has been mentioned before, pCMBS would have decreased P_{os}^c to 0.26 ± 0.17-times the control value.

Apparent activation energy, E_a for P_{os}^c

In these measurements, difficulties arose from at least two factors: the temperature dependence of the permeation process and the temperature dependence of the binding of pCMBS to its receptors. P_{os}^c measurements take a few seconds while drug-receptor equilibration takes much longer (Fig. 3) [17,27]. Accordingly, measurements of P_{os}^c were performed first under control conditions at 37, 27 or 12°C. Then the tubule was exposed to 2.5 mM pCMBS for 25 min (at 10–15°C); the temperature was jumped to one of the values mentioned above within a fraction of a second and P_{os}^c was mea-

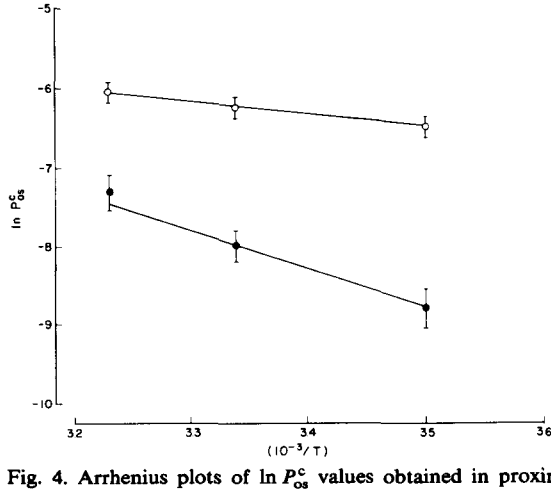

Fig. 4. Arrhenius plots of ln P_{os}^c values obtained in proximal tubules as a function of the reciprocal of the absolute temperature under control conditions (○) and after the preparation had been treated with 2.5 mM pCMBS for 25 min (●). The osmotic difference was 30 mosM. Each experimental point shown is mean ± S.E. of at least 12 measurements.

sured again. By repeating the experiment using different temperature jumps, we got sufficient data to plot ln P_{os}^c vs. the reciprocal of the absolute temperature ($1/T$) (Fig. 4). The slope of the control group of 52 measurements (12 tubules) is clearly different from that obtained after the addition of pCMBS (52 measurements, 12 tubules). From the slopes, the apparent Arrhenius energy of activation for the permeability process was calculated on the assumption that the permeability coefficient could be expressed in the form $P_{os}^c = A \exp(-E_a/RT)$. Where E_a is related to the enthalpy of activation (see Refs. 14 and 22 for a detailed treatment). Under control conditions, E_a was 3.2 ± 1.4 kcal/mol. This value is not statistically different from 4.2 kcal/mol expected for free viscous flow [22]. After the tubule had been treated with pCMBS, E_a significantly increased to 9.2 ± 2.2 kcal/mol.

Action of mersalyl

Addition of mersalyl lowers the values of P_{os}^c. The dose-response curve for the action of mersalyl is shown in Fig. 5. After a 30 min exposure, the $k_{0.5}$ for mersalyl is 0.5 mM. In a series of 12 tubules, 30-min exposure of the tubules to 1 mM mersalyl reduced P_{os}^c significantly to 0.28 ± 0.19 ($n = 12$) of its control value. Subsequent addition of 5 mM dithiothreitol for 10 min reversed this inhibition, yielding a value for P_{os}^c of 1.44 ± 0.21 ($n = 12$) times its control level, a value statistically not different from 1.00.

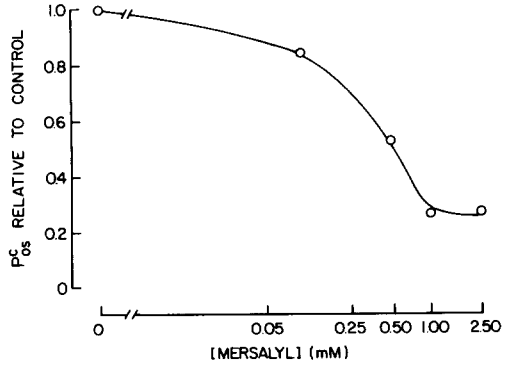

Fig. 5. Dose-response curves showing the action of mersalyl on P_{os}^c. In each experiment after five control measurements of P_{os}^c, the tubules were incubated with mersalyl at the stated concentration for 30 min and P_{os}^c was measured again in the presence of this agent. P_{os}^c is expressed as a fraction of the control value obtained before mersalyl was added. In all measurements, an osmotic difference of 30 mosM was used. Experiments were performed at 10 °C. The graph shows average results.

Mercurial agents react progressively with the sulfhydryl groups existing in the proteins, with very different time constants [27], and mercurial diuretics have their peak of action several hours after administration [31–33]. Therefore, we explored whether exposure to mersalyl for periods longer than the 30 min, used in the dose-response curve for practical reasons, further inhibited P_{os}^c. For this purpose 100 μM mersalyl was added to a tubule and P_{os}^c was measured at 10 °C as a function of time. The experiment was repeated with 12 tubules.

Fig. 3 shows that 100 μM mersalyl has little action after 30 min, in agreement with the dose-response curve (Fig. 5). However, after 50 min, P_{os}^c has in average been reduced to 0.5 of its control value and to 0.2 after 2 h. This action of mersalyl reversed upon addition of dithiothreitol (not shown). The effectiveness of mersalyl at relatively low concentrations in the tubule contrasts with observations in red cells using short exposure times [18]. These results prompted us to explore whether therapeutical concentrations of mersalyl in vivo would show any action on P_{os}^c. We injected 2.4 mg mersalyl (i.e., 5 μmol mersalyl or 2 mg Hg) per kg of body wt. into three different rabbits. Human doses of mercurial diuretics were recommended to be lower than 3.5 mg Hg/kg [31]. The rabbits had free access to water and were killed 2 h later. The tubules were dissected out and mounted to measure P_{os}^c (see Methods). A P_{os}^c value of 0.55 ± 0.15 (relative to control) was obtained in 32 tubules, significantly different from 1.00 ($p < 0.001$). Subsequent in vitro addition of 5 mM dithiothreitol for 10 min increased the ratio to a value of 0.79 ± 0.13 ($n = 32$), a value not statistically different from 1.00.

Discussion

The main findings of this work are: (1) that under control conditions, P_{os}^c varies with temperature as expected from the changes in the water viscosity in free solution. This leads to calculation of E_a for P_{os}^c of 3.2 ± 1.4 kcal/mol. (2) The use of

2.5 mM of the sulfhydryl reagent pCMBS lowers P_{os}^c from a control value (extrapolated to 0 osmotic difference) of $50.4 \cdot 10^{-4}$ cm^3/s per osM per cm^2 of basal membrane area to 0.26 of this value, i.e., to $13.1 \cdot 10^{-4}$ in the same units. As mentioned in Introduction, pCMBS was chosen because of its specificity on water transport [25] and because it is known to penetrate only very slowly and to a small degree into the cell interior [34] so that the possibility that it would affect other cell processes inside the cell are less than with other mercurial sulfhydryl reagents [3]. Mersalyl has a similar action. This action of pCMBS and mersalyl on P_{os}^c has the following characteristics: (a) it is dose-dependent; (b) it is quickly reversible upon addition of dithiothreitol; (c) the action of pCMBS can be prevented by the use of thiourea; (d) therapeutic intramuscular doses of mersalyl significantly reduce P_{os}^c. This action can be reverted by subsequent addition of dithiothreitol.

The observation that the action of pCMBS and of mersalyl is reversible upon addition of dithiothreitol indicates that sulfhydryl groups are involved in the structure of the water pathways, and that the reduction of P_{os}^c by the sulfhydryl reagents is neither due to damage of the preparation during the incubation period nor to an irreversible effect of the mercurial compounds on the membrane. On the contrary, its quick reversibility with dithiothreitol indicates easily accessible sites of action for pCMBS and mersalyl.

Prevention of pCMBS action by thiourea has been observed in red cells [20]. It probably indicates that thiourea in kidney cell membranes, as in erythrocytes, acts near the site of action of pCMBS. However, the high concentrations of thiourea needed indicate a low degree of affinity for this action.

Action of pCMBS on P_{os}^c and on its E_a

The observation that E_a for P_{os}^c is 3.2 kcal/mol under control conditions to be compared with 4.2 kcal/mol, which is the E_a for bulk water viscosity, indicates that a continuum for water movement must exist across the cell membrane, thus explaining a low degree of interaction between the water molecules, moving across the membrane under the osmotic force, and the membrne. The possibility that P_{os}^c is apparently less (though not necessarily significantly so) than 4.2 kcal/mol would be compatible with some slip at the water-membrane interface in the water pore, in contrast to the zero velocity at the interface layer in Poiseuille flow in glass capillaries in which viscosity is normally measured. In these respects, the present observations resemble those made in untreated (control) erythrocytes [22]. As was previously reported in red cells, E_a increases significantly to 9.2 kcal/mol after addition of pCMBS. If filtration of water across the tubular cell membrane were, as in lipid bilayers, without water channels, E_a should be near 10 kcal/mol or larger. This indicates that the interaction between water molecules crossing the membrane and the proximal tubular cell membrane is increased by pCMBS as compared to that observed under control conditions.

In additon, the P_{os}^c values in the presence of pCMBS are about one-fourth of the control values. P_{os}^c is evaluated from the volume flow induced per unit osmotic concentration difference. If the pathways through which the water movement occurs are cylindrical *, $P_{os}^c = N\pi r^4/8\eta\Delta x$, where N is the number and r the radius of each of these pathways, η is the water viscosity within the pathways (and should reflect the degree of interaction between water and membrane) and Δx the length of each of these pathways. Therefore, P_{os}^c depends on geometrical factors and on η. The values for E_a of P_{os}^c under control conditions indicates that η may be as in free solution *. The decrease in P_{os}^c with pCMBS must be due to geometrical terms since addition of pCMBS does not change the viscosity of water. This must, in all probability, be due to conformational changes in the geometry of the aqueous pathways (which leads to their virtual occlusion through highly specific interaction of pCMBS with sulfhydryl groups in the membrane protein related to the structure of the pore), rather than by mechanical blocking of the pore by the

* The validity of a calculation of pore radius based on the continuum hydrodynamic theory has been checked experimentally only for pores several nm in radius [20]. If the pathways are not exactly cylindrical, the geometrical factors change, but this is immaterial for the calculations that follow which are to be taken as an example to analyze the significance of the effect of pCMBS on P_{os}^c and not as an actual representation of the shape of the water pathway in the membrane.

pCMBS molecule itself. This change need be only small, since r intervenes as r^4. Dithiothreitol reverts this change and suggests that the main action of pCMBS is not secondary to irreversible changes or to reaction of this agent with groups other than sulfhydryl. Still unexplained is the observation that although the effect of pCMBS on water transport has a time-course in the order of 30 min, the reversal of pCMBS inhibition by dithiothreitol is of the order of 5 min.

Some tentative calculations

With the equation given in the last paragraph and the present values of P_{os}^c of $50.4 \cdot 10^{-4}$ cm^3/s per cm^2 of basal membrane osM, we can calculate the number of cylindrical pores, N, that might pierce the kidney cell membrane, if we use $\eta = 0.69$ cP (the viscosity of water in free solution at 37 °C, equivalent to $2.69 \cdot 10^{-10}$ osM/s) and $\Delta x = 7.5$ nm. We assume that r ranges from 0.35 to 0.5 nm which would include pores the size of the gramicidin channel and the value of r measured in red cells [12,20]. N turns out to range from 0.4 to $1.7 \cdot 10^{12}$ pores/cm^2 of basal membrane area, which would occupy less than 1% of the tubular area. Due to the basolateral infoldings, there are 8.6 cm^2 of real basolateral cell membrane areas per cm^2 of basal membrane area [35]. Therefore, P_{os}^c is $5.9 \cdot 10^{-4}$ cm^3/s per cm^2 of real basolateral cell membrane area per osM and $(5–20) \cdot 10^{10}$ pores occupy 1 cm^2 of real basolateral cell membrane area. These values are to be compared with a figure of $1.7 \cdot 10^{11}$ pores/cm^2 of erythrocyte area. There are $2.7 \cdot 10^5$ pores/red cell. The average area of a red cell is 163 μ^2 [20]. In our preparation, the water osmotic permeability per pore turns out to range from $(3–12) \cdot 10^{-15}$ cm^3/s per osM, to be compared with figures of the order of $(1.7–2.5) \cdot 10^{-15}$ (same units) for the red cell and of $1.0 \cdot 10^{-15}$ for the gramicidin channel [12]. Given the number of assumptions used in the calculations, the coincidence between these water permeabilities may be fortuitous. However, agreement between red cells and proximal tubular cells might be expected, since the water osmotic permeability of these two cells agree when expressed per real cell surface area [8].

In short, the present paper shows high values of P_{os}^c in the peritubular cell membrane of untreated proximal straight tubule with an E_a compatible with free water movements, as observed in viscous flow, strongly suggesting the presence of continuous water pathways piercing the cell membrane. pCMBS lowers P_{os}^c and increases E_a to 9.2 ± 2.2 kcal/mol. Whatever permeability remains in the presence of pCMBS, it can be due to the presence of a diffusive flow driven by the difference in water activity set up by the osmotic gradient experimentally imposed between bath and cell interior, and to the presence, if any, of some parallel remnant viscous flow. This latter possibility is suggested, because E_a in the presence of pCMBS falls a bit short of the 12–14 kcal/mol expected from bilayers.

Besides the evidence presented in this paper, and as mentioned in Introduction, the following additional criteria help define the presence of aqueous pores and therefore of viscous flow in cell and artificial membranes. (a) The observation that P_{os}/P_d for water is significantly larger than 1; (b) the demonstration of solvent drag of solute accompanying the osmotic flow; (c) the presence of graded permeability to small solutes (molecular sieving); (d) the observation that the ratio of the diffusive permeability for water to that for other solutes greatly exceeds the values for such ratios in artificial membranes [23]. This ratio keeps a fairly constant value for a given lipid bilayer (not containing water pores) and is greatly exceeded when these membranes are incorporated with pre-forming antibiotics [23]. Experiments along these lines should be tried in the kidney tubule to further the present findings and to ascertain whether the aqueous pores are also used by small non-electrolytes and ions [9].

Another remaining question relates to the protein responsible for these water pathways. Evidence that Band III is related to the water pore of red cells has been provided, although these are still conflicting view [20]. Measurements of this protein density in kidney tubular cell membranes should lead to finding an answer to this question.

Finally, a word about the action of mercurial diuretics. Although there is evidence that mercurial compounds inhibit salt transport in epithelia, and this explains the diuretic action of these compounds [31–33,36], the present experiments open the possibility that curtailment of water permeability contributes to their diuretic effect.

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