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Osmotic water permeability and solute reflection coefficients of rat kidney brush-border membrane vesicles

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Solute reflection coefficients, σ_i, of rat kidney brush-border membrane vesicles were determined by the comparison of water flows induced by equiosmolal gradients of sucrose and NaCl, KCl or mannitol. The values of 0.53 for σ_{NaCl} and 0.56 for σ_{KCl} when compared with 0.92 for $\sigma_{mannitol}$ suggested some interactions between salt and water pathways. Altering the membrane proteins with 0.4 mM $HgCl_2$ decreased the osmotic water permeability of the vesicles by 70 to 80% and brought σ_{NaCl} and σ_{KCl} to a value not different from 1. This argued in favor of water protein pathways in the luminal membrane of kidney proximal cells which are partly accessible to NaCl and KCl.

The water permeability of the rat kidney brush-border membranes calculated from the rate of shrinkage of vesicles submitted to an osmotic gradient was close to 240 μm/s [1]. This high value could be reduced by 60 to 80% by altering the membrane proteins with sulfhydryl reagents such as *para*-chloromercuribenzenesulfonic acid (PCMBS) or mercuric chloride. This inhibition was not observed with non-mercuric sulfhydryl reagents and was reversible by addition of cysteine to the incubation medium [1].

The dependence of fast osmotic water fluxes on membrane proteins integrity suggested that there were some proteic water pathways in the luminal membranes of rat kidney proximal tubules which facilitated the movement of water molecules. This hypothesis was also supported by the data of Berry [2] and Preisig and Berry [3] who reported that intraluminal perfusion of PCMBS reduced the transepithelial diffusion of tritiated water across the proximal tubule of rabbit or rat kidney. Whether such water pathways are also shared by salts or are only accessible to water molecules has not yet been investigated. Complete independence between solute and water pathways implies that all solutes exhibit comparable water fluxes at identical osmotic gradients, i.e. that the solute reflection coefficient σ_i defined by Staverman [4] is close to 1 for kidney brush-border membrane. The alternative, interaction between salt and water channels, corresponds to σ_i lower than 1, and blockage of the expected common transmembrane pathways by sulfhydryl reagents would modify the salt reflection coefficients. This approach has been successfully used by Chasan and Solomon [5] to demonstrate interaction between urea and water pathways across the red blood cell membrane.

Solute reflection coefficients of rat kidney brush-border membrane vesicles were determined in the present study by comparing the water fluxes induced by isoosmolal gradient of sucrose, mannitol, NaCl and KCl with or without $HgCl_2$ in the incubation medium.

Renal brush-border membranes were prepared

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by the calcium precipitation method from male Sprague-Dawley rats weighing 280–320 g, and were sealed in a buffer solution containing 50 mM sucrose, 10 mM Hepes Tris (pH 7.5) and 0.01% NaN_3. The osmotic pressure of this control buffer solution was close to 75 mosmol/kg H_2O; hyperosmolal mixing solutions were made by the addition of the required amounts of sucrose, mannitol, NaCl or KCl to the control solution to give final osmotic gradients of 50–100 or 150 mosmol/kg H_2O in the optical cell. Osmolalities were checked with a vapor pressure osmometer (Wescor). Kinetic measurements of vesicle shrinkage were performed with a stopped-flow apparatus coupled to light transmission recording as previously described [1]. Changes in light intensity were measured over a 1000 ms time interval starting 3 ms after mixing the vesicles with hyperosmotic solution. Three runs were usually stored and averaged at each given condition from every experiment. Since the size of the vesicles was small (mean diameter 0.137 μm), unstirred layer effects are minimum and initial slope of change in light intensity is directly accessible for analysis. All the experiments were performed with freshly prepared vesicles and the temperature was 25°C.

Because sucrose is barely permeable across the brush-border membrane vesicles [1], it was considered that it exerts its full osmotic gradient and consequently that its reflection coefficient σ_s is equal to one. The σ_i for the i solute was thus calculated from the ratio of initial water fluxes induced by same gradient of sucrose and of the given solute: $\sigma_i = JV_i/JV_s$ at identical osmolality [4]. Considering that under the present conditions of light transmission recording, the initial change in light intensity $(dI/dt)(0)$ extrapolated from the fit of the experimental curve of transmitted light intensity as a function of time was proportional to $(dV/dt)(0)$

$$\sigma_i = \frac{\frac{dI}{dt}(0)_i}{\frac{dI}{dt}(0)_s}$$

The comparison of the obtained $(dI/dt)(0)$ for sucrose and NaCl at three different osmotic gradients is shown in Fig. 1. The initial rate of vesicle shrinkage was linearly dependent on the imposed

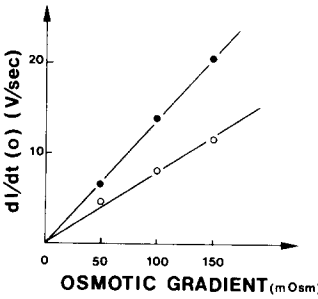

Fig. 1. Relationship between initial change in light transmission intensity $(dI/dt)(0)$ and initial osmotic gradient in one of four experiments. Reflection coefficient for NaCl was calculated from the paired comparison of these slopes. In this experiment σ_{NaCl} was 0.59. ●, sucrose; ○, NaCl.

osmotic gradient for both sucrose and NaCl, but for identical osmotic gradient, the rate of shrinkage was less with NaCl than with sucrose. The NaCl reflection coefficient, σ_{NaCl}, calculated from the ratio of the slopes depicted in Fig. 1 was 0.57 ± 0.01 ($n = 4$), a value significantly different from 1. This value was close to the 0.53 ± 0.02 ($n = 5$) calculated from the $(dI/dt)(0)$ ratio at the single osmotic gradient of 150 mosmol/kg H_2O. Accordingly, in the following experiments, σ_i were determined from the $(dI/dt)(0)$ ratio at this 150 mosmol/kg H_2O gradient. The KCl and mannitol reflection coefficients, σ_{KCl} and $\sigma_{mannitol}$, measured in this way are reported in Table I. Whereas

TABLE I

SOLUTE REFLECTION COEFFICIENTS OF RAT KIDNEY BRUSH-BORDER MEMBRANE VESICLES DETERMINED IN THE PRESENCE AND ABSENCE OF 0.4 mM MERCURIC CHLORIDE

Reflection coefficients are calculated from the ratio of the initial slope of change in light transmission intensity due to an osmotic gradient of 150 mosmol/kg H_2O sucrose over 150 mosmol/kg H_2O NaCl, KCl or mannitol. Values are means ± S.E., n is the number of experiments.

	Solute reflection coefficients	
	Control	+0.4 mM $HgCl_2$
NaCl ($n = 5$)	0.53 ± 0.02	1.01 ± 0.13 *
KCl ($n = 5$)	0.56 ± 0.03	0.91 ± 0.09 *
Mannitol ($n = 3$)	0.92 ± 0.03	0.90 ± 0.10

* $P < 0.05$. Statistical significance was tested with the nonparametric Wilcoxon procedure.

σ_{KCl} was comparable with σ_{NaCl}, thus indicating similar interactions with water pathways for the two ionic species, $\sigma_{mannitol}$ was close to one. From these reflection coefficient measurements, it would appear that NaCl and KCl, but not mannitol, partly share a common pathway with water molecules. It also suggests that the equivalent pore radius of these pathways is equal or smaller than 4.4 Å, the molecular radius of hexose [6].

Preincubation of the brush-border membrane vesicles with 0.4 mM $HgCl_2$ for 15 min reduced the osmotic water permeability by 73 to 80% (mean = 77 ± 1%, $n = 5$). The comparison of $(dI/dt)(0)$ induced by equiosmolal gradients of sucrose, NaCl and KCl when water proteic pathways are altered by mercuric chloride, revealed identical initial rates of vesicle shrinkage (Fig. 2, Table I). Using the same calculations as in the absence of mercuric chloride it appeared that shutting most of the water pathways brought the σ_{NaCl} from 0.53 to a value not significantly different from 1 (Table I). $\sigma_{mannitol}$ which was already close to 1 in control conditions remained unchanged by the presence of the sulfhydryl reagent (Table I). This suggests that mercuric chloride alters the aqueous channels which interact with NaCl and KCl, leaving residual water pathways independent on those of salts.

The measurement of a σ_i lower than 1 for salt differs from the lack of solvent drag reported on rabbit proximal tubule perfused in vitro [7,8]. Apart from the species difference, rat as opposed to rabbit, or from possible changes in the permeabilities of brush-border membranes due to the technique of their isolation, a possible explanation of this disagreement is that the reflection coefficient of the entire cells is a function of the reflective properties of the luminal and basolateral membranes in series. In this context, the report of Gonzáles et al. [9] indicating that σ_{NaCl} of the basolateral membrane of rabbit proximal tubule is not different from 1 would support the idea that no solvent drag occurs across kidney proximal tubule although the salt reflection coefficient of its luminal membrane is lower than 1.

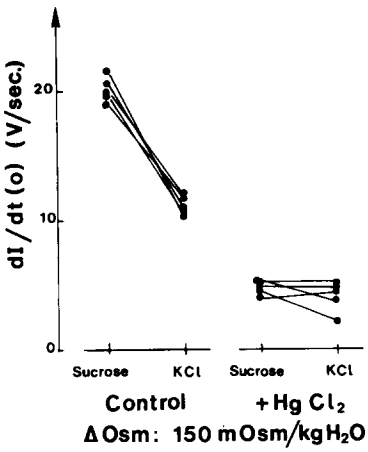

Fig. 2. Initial slopes of changes in light transmission intensity $(dI/dt)(0)$ induced by isoosmolar amount of sucrose and KCl. Data from a same experiment are joined by line. KCl reflection coefficients calculated from these numbers were 0.56 ± 0.03 in control condition and 0.91 ± 0.09 in the presence of $HgCl_2$. The addition of the sulfhydryl reagent $HgCl_2$ reduced the osmotic water permeability of the brush-border membrane vesicles by 77%.

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