Biochimica et Biophysica Acta, 436 (1976) 242—246

BBA Report

BBA 71255

PERMEABILITY OF RABBIT POLYMORPHONUCLEAR LEUKOCYTE MEMBRANES TO UREA AND OTHER NONELECTROLYTES

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(Received March 16th, 1976)

Summary

The diffusional permeability coefficients of rabbit polymorphonuclear leukocyte membranes to urea, methylurea and thiourea have been measured. It was found that the permeability coefficient of these membranes to urea is very low and that thiourea was more permeable than methylurea which was, in turn, more permeable than urea. These results suggest that there is no need to postulate a carrier-mediated mechanism for urea transport across biological membranes and that the concept of "aqueous pores" is not a general property of biological membranes but restricted only to certain cases.

The transport of urea across various mammalian red cell membranes has been studied extensively [1—6]. The mechanism of urea transport in these cells has been subject to considerable debate. This controversy stems from the fact that urea is a small hydrophilic molecule which is able to form with water 5 hydrogen-bonds, and yet, in spite of these properties, it is extremely permeable across red cell membranes. The simplest and most straight-forward explanation for this is to postulate that the red cell membrane behaves operationally as a mosaic structure containing both lipid and polar regions. According to this, small hydrophilic solutes permeate through the polar routes. An alternative explanation is to postulate that urea moves across biological membranes by means of a specialized carrier-mediated mechanism. Since there are consistent sets of arguments for the presence of aqueous pathways in mammalian red cell membranes, it is not possible to decide which of these two mechanisms is responsible for the high rate of urea transport. The present studies were undertaken for two reasons. First, no data is available about the transport of urea in polymorphonuclear leukocyte membranes. Second, the value of the hydraulic permeability coefficient of these membranes to water is much lower than the

corresponding value for red cells and also the value of the apparent activation energy for water transport in the former cell membrane is much higher than the value for the latter membrane [7, 8]. These two findings suggest that rabbit polymorphonuclear leukocyte membrane does not act as a molecular sieve (absence of equivalent pores). Accordingly, it is possible, using these membranes, to decide between these two possible mechanisms for urea transport. For example, if urea is transported across biological membranes by a carrier-mediated mechanism, then the rate of urea movement across polymorphonuclear leukocyte membranes would be very high. On the other hand, if urea permeates through "aqueous pores", then the permeability coefficient of these membranes to urea would be very small. Furthermore, the rates of permeation of urea, methylurea and thiourea, across polymorphonuclear leukocyte membranes would be determined mainly by the lipid solubility of each solute.

We have found that the permeability of rabbit leukocyte membranes to urea is very low and that thiourea was more permeable than methylurea which was more permeable than urea. This is the first reported case among membranes from mammalian species where thiourea permeates faster than urea.

Polymorphonuclear leukocytes were obtained from white albino rabbits (5—7 lb) which were injected intraperitoneally with 300—500 ml of isotonic sterile solution containing glycogen (0.5 g/l). The peritoneal exudate was collected 16 h later in a heparinized flask. The leukocyte-rich exudate was strained through four layers of cheesecloth to remove large clumps of debris. The suspension was gently centrifuged at 500 x *g* and then the supernatant was removed and replaced by equal volumes of isotonic buffered NH_4Cl. The packed cells were resuspended with a Pasteur pipette and kept at room temperature for 5 min. This procedure was necessary to hemolyze red cells [7]. The suspension was centrifuged for 5 min at 500 x *g*, and the cells were immediately washed twice with a buffered isotonic solution and then resuspended (20×10^6 cells/ml). A known volume (0.3 ml) of the suspension was layered on top of a silicone oil layer (F50, density 1.05 g/cm^3) in 1.5 ml capacity microcentrifuge tubes. 0.2 ml of radioactive buffered solution was injected into the suspension and the sample was centrifuged after a preset time had elapsed. Mixing was achieved by injecting the large volume of the buffered solution containing the radioactivity. The separation of the cell from the suspending media was accomplished by a single centrifugation (0.5 min) in an Eppendorf microcentrifuge. The cells were separated in less than 10 s. The supernatant and the oil layer were removed by suction and the pellets were solubilized in 0.1 M NaOH in 2% Na_2CO_3 at 50°C. A sample was removed for protein determination and another sample for counting. It was found that practically no radioactivity could be found in the oil layer (1% of the total count in the pellet of the first sample). The trapped extracellular space, as determined by [^{14}C]-inulin, was less than 5% of the total counts in the pellet of the first sample.

Fig. 1 shows the time course of [^{14}C]urea uptake by rabbit polymorphonuclear leukocyte membranes at 25°C. In all experiments the cells were initially equilibrated with 1 mmol of unlabelled urea before the radioactivity was added. Fig. 2 shows the results of another experiment. The data plotted in this manner fall on a straight line between 10 and 60 s, consistent with two-

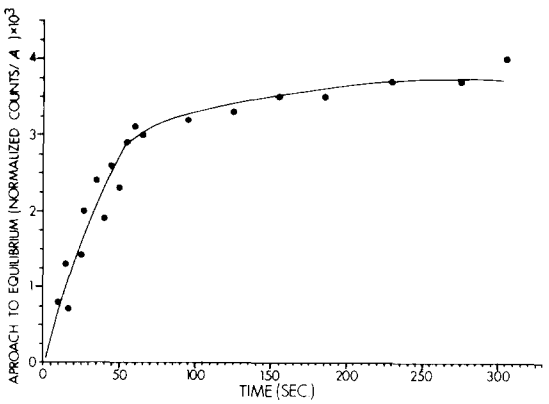

Fig. 1. The time course of [^{14}C]urea uptake by rabbit polymorphonuclear leukocyte membranes at 25° C

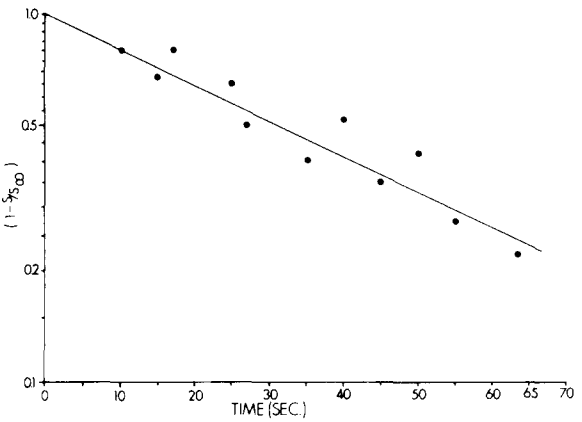

Fig. 2. The time course of [^{14}C]urea uptake by rabbit polymorphonuclear leukocyte membranes at 25° C
S and S_∞ represent the counts/absorbance at any given time and at equilibrium respectively.

TABLE I

PERMEABILITY COEFFICIENTS OF RABBIT POLYMORPHONUCLEAR LEUKOCYTE MEMBRANES TO UREA

For the permeability coefficient, the volume of cell water, V, is equal to $233 \cdot 10^{-12}$ cm^3 [7]. The surface area (A) calculated assuming that the sphere is equal to $259 \cdot 10^{-8}$ cm^2.

Experiment number	Slope (s^{-1})	Half-time (s)	Permeability coefficient (cm/s) x 10^5
1	−0.033	21	0.42
2	−0.020	35	0.26
3	−0.017	41	0.22
4	−0.034	20	0.45
5	−0.019	36	0.25
6	−0.024	29	0.31
7	−0.024	29	0.31
8	−0.018	38	0.24
Mean ± S.E.M.		31 ± 4	0.30 ± 0.03

compartment kinetics. The half-time of the exchange, $t_{1/2}$, may be obtained by setting $s = \frac{1}{2}S_\infty$. Thus, $t_{1/2} = -0.693/\text{slope}$. A number of determinations of the rate constant for urea transport in these cells are summarized in Table I. The

average value of $t_{1/2}$ for urea transport in polymorphonuclear leukocyte membranes is 31 ± 4 s, which is much larger than the value of 53 ± 6 ms for human red cell membranes. This implies that the permeability coefficient for urea is at least two orders of magnitude smaller in the former cells than the corresponding value in the latter cells. If one assumes that a carrier-mediated mechanism is responsible for the extremely high permeability of human red cell membrane to urea, then one would be forced to conclude that such a mechanism is absent in rabbit polymorphonuclear leukocyte membranes. It is most likely that no such mechanism for urea transport exists in biological membranes. The difference in the behavior of the two membranes to urea transport can be accounted for on the basis of the presence or absence of aqueous pores. The simplest explanation would be to postulate that red cell membranes behave operationally as a selective solvent and a molecular sieve (containing aqueous pores), whereas polymorphonuclear leukocyte membranes act only as a selective solvent (do not contain pores).

One of the basic findings in biological membranes which are characterized by the presence of "aqueous pores" is that the permeability coefficients of these membranes to small hydrophilic nonelectrolytes are much higher than predicted on the basis of the lipid solubility of these solutes. For example, the permeability coefficient of human red cell membrane to urea is 7 times greater than the corresponding value for methylurea, although the value for the K_{ether} of latter solutes is 2.5 times greater than the corresponding value for the former. Furthermore, the permeability coefficient of the same membrane to urea is 3 orders of magnitude higher than the corresponding value for thiourea even though the K_{ether} is one order of magnitude higher for thiourea. If rabbit polymorphonuclear leukocyte membranes do not contain "aqueous pores", then one would predict that its permeability coefficient to these molecules would be determined by three factors: the lipid solubility of the solute, its shape and size, and the number of hydrogen bonds, N_H, it is able to form with water. To test this, we measured the permeability coefficients of rabbit polymorphonuclear leukocyte membranes to urea, methylurea and thiourea. The results are shown in Table II. The values for K_{ether}, N_H and molar volume are included in the table. The permeability coefficients of human red cell membranes to these solutes are also included in the table for comparison. It is very clear from the table that the two membranes behave quite differently with respect to the rates of permeation of these nonelectrolytes. This difference

TABLE II

PERMEABILITY COEFFICIENTS OF RABBIT POLYMORPHONUCLEAR LEUKOCYTE (PMN) AND HUMAN RED BLOOD CELL (RBC) MEMBRANES TO SMALL NONELECTROLYTES

Solute	Molar vol.[a]	K_{ether}[b]	N_H	Permeability coefficient x 10^5 (cm/sec)	
				Rabbit PMN	Human RBC
Urea	45	0.00047	5	0.30 ± 0.03 (8)[c]	31.2[d]
Methylurea	61.5	0.0012	4	0.45 ± 0.05 (3)	4.8[d]
Thiourea	54.2	0.0063	5	0.87 ± 0.10 (3)	0.07[e]

[a] Molar volume is expressed in terms of $cm^3 \cdot mol^{-1}$.
[b] Values are taken from ref. 10.
[c] Number in parentheses refers to the number of experiments.
[d] Values are taken from ref. 2.
[e] Values are taken from ref. 11.

cannot be accounted for on the basis of differences in species. The pattern in the permeability coefficients of rabbit red cell membrane to urea, methylurea and thiourea is similar to that found for human erythrocyte membranes.

Three conclusions can be drawn from these studies. First, there is no need to postulate a specialized mechanism (carrier mediated) for urea transport across rabbit polymorphonuclear leukocyte membranes. This conclusion can be extended probably to human red cell and other biological membranes. It is very difficult to imagine why red cell membranes would have a carrier mediated mechanism for urea transport and not polymorphonuclear leukocyte membranes. Second, there is no need to postulate that polymorphonuclear membranes contain "aqueous pores" for the transport of small hydrophilic nonelectrolytes. In these cells one can postulate that the membrane is homogeneous and permeation occurs only by dissolution in the membrane fabric. Third, the idea of "aqueous pores" is not a general property of biological membranes but restricted only to certain membranes.

This work was supported in part by a grant from the National Institute of Health GM 20268-02.

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