Role of Facilitative Glucose Transporters in Diffusional Water Permeability Through J774 Cells

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ABSTRACT We have reported previously that in the presence of an osmotic gradient, facilitative glucose transporters (GLUTs) act as a transmembrane pathway for water flow. Here, we find evidence that they also allow water passage in the absence of an osmotic gradient. We applied the linear diffusion technique to measure the diffusional permeability (Pd) of tritiated water (3H2O) through plasma membranes of J774 murine macrophage-like cells. Untreated cells had a Pd of 30.9 ± 1.8 μm/s; the inhibitors of facilitative glucose transport cytochalasin B (10 μM) and phloretin (20 μM) reduced that value to 15.3 ± 1.8 (50%) and 11.0 ± 0.7 (62%) μm/s, respectively. In contrast, no significant effect on Pd was observed in cells treated with dihydrocytochalasin B (Pd = 28.4 ± 1.5 μm/s). PCMBS (3 mM) inhibited glucose uptake by greater than 95%, and 3H2O diffusion by ~30% (Pd = 22.9 ± 1.5 μm/s). The combination of cytochalasin B plus PCMBS reduced Pd by about 87% (Pd = 3.9 ± 0.3 μm/s). Moreover, 1 mM PCMBS did not affect the osmotic water permeability in Xenopus laevis oocytes expressing the brain/erythroid form of facilitative glucose transporters (GLUT1). These results indicate for the first time that about half of the total Pd of J774 cells may be accounted for by water passage across GLUTs. Hence, they highlight the multifunctional properties of these transporters serving as conduits for both water and glucose. Our results also suggest for the first time that PCMBS blocks glucose transport without affecting water permeation through GLUTs. Lastly, because PCMBS decreases the Pd of J774 cells, this suggests the presence in their plasma membranes of another protein(s) exhibiting water channel properties.

INTRODUCTION

Water movements across membranes are fundamental in biology (Stein, 1986; Finkelstein, 1987). Because bilayers of lipids extracted from cell membranes exhibit...
much less variation in water permeability than the membranes from which these lipids were obtained, it has been suggested (Stein, 1986; Finkelstein, 1987), that cellular membranes express proteins that facilitate the translocation of water across membranes. Preston and Agre (1991) identified and cloned an integral membrane protein of erythrocytes (CHIP28), and showed that it is a water channel (Preston, Carroll, Guggino, and Agre, 1992) sensitive to mercurials. Other proteins such as facilitative glucose transporters (Fischbarg, Kuang, Hirsch, Lecuona, Rogozinski, Silverstein, and Loike, 1989; Fischbarg, Kuang, Vera, Arant, Silverstein, Loike, and Rosen, 1990; Zhang, Alper, Thorens, and Verkman, 1991), Na⁺ glucose cotransporters (Loike, Arant, Kuang, Xu, Cao, Silverstein, and Fischbarg, 1991) and the cystic fibrosis transmembrane conductance regulator (Hasegawa, Skach, Baker, Calayag, Lingappa, and Verkman, 1992) have also been shown to act as conduits for water driven osmotically across membranes.

We examine here water permeation in J774 cells in the absence of an osmotic gradient. We employed the linear diffusion method (Redwood, Rail, and Perl, 1974; Garrick, 1989; Crank, 1957) to measure diffusional water permeability (Pd) across the plasma membranes of a column of packed J774 cells. Inhibitors of facilitative glucose transport significantly reduce Pd in these cells. pCMBS reduces Pd values by about 30% and glucose uptake by greater than 95% in J774 cells, but does not affect osmotic water permeability in Xenopus laevis oocytes expressing the brain/erythroid glucose transporter (GLUT1). Our results suggest that GLUTs act as conduits for diffusional water movements, and also suggest the presence of additional protein(s) with water channel properties in the membranes of J77 cells.

MATERIALS AND METHODS

Cell Culture

J774 cells were maintained as suspension cultures at 15 x 10⁶ cells/ml in Dulbecco’s modified Eagle’s medium containing 5 mM glucose, 10% (vol/vol) heat-inactivated calf serum, and penicillin/streptomycin (DMEM+) as described (Fischbarg et al., 1989). Cell diameters were measured by phase microscopy-split screen micrometry using suspended cells that had been fixed for 10 min in phosphate-buffered saline containing 3.7% paraformaldehyde. The cell average diameter was 21.2 ± 0.4 μm (n = 100). Reagents and drugs were from Sigma Chemical Co. (St. Louis, MO); cell culture media and sera were from Gibco Laboratories (Grand Island, NY); radioisotopes were from New England Nuclear (Boston, MA).

Diffusion Studies

The Pd of J774 cells was measured as described (Redwood et al., 1974; Garrick, 1989; Crank, 1957). Cells were concentrated to 20–25 x 10⁶ cells/ml and resuspended in 0.5 ml of DMEM+. More than 90% of the resuspended cells remained viable as determined by trypan blue exclusion (Loike, Kozler, and Silverstein, 1979).

10 μl of ¹⁴C-dextran (0.1 μCi/μl) was added to 0.1 ml of cell suspension for determination of extracellular space. This labeled suspension was drawn into a 6 cm piece of polyethylene tubing (PE205, ClayAdams, Parsippany, NJ). One end of the tube was sealed with soft clay and fused with heat (distally to the clay plug). These sealed tubes were centrifuged at 500 g for 10 min at room temperature to pack the cells further. The polyethylene tubes were then sliced at two sites
(5 mm below the cell-medium interface, and just above the cell-medium interface). The portion of tubing above the cell-medium interface (which contained DMEM+) was frozen with solid CO$_2$ and was cut into five slices of 1 mm thickness, which were immediately transferred into scintillation vials. The $^{14}$C-dextran in the medium in this portion of the tube served to measure extracellular space. The portion of tubing between the cell-medium interface and 5 mm below that interface was discarded because it usually contained imperfectly packed cells. The remaining segment of tubing was then placed into a glass test tube at 37°C. After 5 min, 23 μl (12 μCi/μl) of $^3$H-H$_2$O (specific activity = 1.0 mCi/mmol) was added to the open end of the cell column. After 2 min, the excess liquid at the open end was removed by blotting, and diffusion of $^3$H-H$_2$O was allowed to proceed. After 30 min, the tube containing the packed cells was frozen with solid CO$_2$ and sliced from the open end at 1 mm intervals (total: 10 slices). $^3$H and $^{14}$C radioactivity in these slices was determined in an LKB liquid scintillation counter.

In other experiments, diffusion of $^3$H-H$_2$O through the medium was measured as described (Garrick, 1989). To determine the intracellular diffusion coefficient, J774 cells were sedimented in polyethylene tubes as described above, and were lysed by subjecting to three cycles of freezing and thawing.

Coefficients for water diffusion through the column of packed viable cells ($D$), through extracellular medium ($D_1$), and through intracellular medium [cells that were frozen and thawed repeatedly; $D_2$], were determined as described (Garrick, 1989). Numerical values of the diffusional water permeabilities through the packed columns of cells were obtained using the experimental values of $D$, $D_1$, $D_2$, relative extracellular (dextran) space ($V_1/V = 0.11$ to 0.14; $V_1$ and $V$ as in Garrick [1989]) and our measured average diameter for J774 cells, and substituting those values into the appropriate equations as described (Crank, 1957; Redwood et al., 1974; Garrick and Redwood, 1977; Garrick, 1989). Deviations are SEM.

**Expression of GLUT1 in Oocytes**

_Xenopus laevis_ oocytes were prepared as described by Milovanovic, Frindt, Tate, and Windhager, 1991. Oocytes were injected with either 50 nl of water (control) or with 50 ng of GLUT1 cRNA in 50 nl of water. All measurements of osmotic water permeability ($P_l$) were conducted at room temperature. An oocyte was transferred from culture medium into a glass chamber with Barth's solution (Echevarría, Kuang, Iserovich, Li, Preston, Agre, and Fischbarg, 1993) and left undisturbed for some 30 min. Controls (not shown) indicated that such interval was sufficient for the oocyte volume to reach a steady state. The oocyte was then exposed to a 92% hypotonic challenge. The change in oocyte volume was monitored and its $P_l$ was calculated as described (Fischbarg et al., 1990; Echevarría et al., 1993).

**RESULTS**

**Diffusion and Permeability Coefficients for $^3$H–H$_2$O**

The mean values of the diffusion coefficients for $^3$H-H$_2$O ($D_1$, $D_2$, and $D$) are given in Table I. The diffusion coefficient for $^3$H-H$_2$O through packed cells ($D = 3.98 \times 10^6$ cm$^2$/s) is considerably lower than the corresponding values for the frozen and thawed cells (i.e., intracellular; $D_2 = 12.4 \times 10^6$ cm$^2$/s) or cell-free medium (i.e., extracellular; $D_1 = 23.8 \times 10^6$ cm$^2$/s) media. As expected, an intact plasma membrane generates a marked resistance to water diffusion.
TABLE I

Diffusion Coefficients at 37°C for 3H-H2O in J774 Cells

|                | D1        | D2        | D          |
|----------------|-----------|-----------|------------|
| Control        | 23.8 ± 0.4 (2) | 12.4 ± 1.0 (31) | 3.98 ± 0.24 (25) |
| Phloretin (20 μM) | 10.8 ± 1.8 (12) | 2.64 ± 0.17 (13) |            |
| Cytochalasin B (10.0 μg/ml) | 11.5 ± 1.8 (11) | 2.96 ± 0.36 (10) |            |
| Dihydrocytochalasin B (10 μg/ml) |          | 3.80 ± 0.20 (6) |            |
| pCMBS (3 mM)   | 16.5 ± 1.7 (6) | 3.50 ± 0.19 (16) |            |

Numbers of experiments in parenthesis. One experiment represents one tube of packed cells. D: water diffusion through the column of packed cells; D1: through extracellular medium; and D2: through lysed cells.

Glucose Transport Inhibitors

J774 cells were preincubated for 30 min at 37°C in the presence of cytochalasin B (CytB; 10 μM) or phloretin (Phi; 20 μM). Subsequently, the cells were packed into polyethylene tubes, and the diffusion of 3H-H2O was measured at 37°C for up to 30 min. The 3H-H2O diffusion coefficient in CytB- or Phi-treated cells was reduced by ~26% and 34%, respectively (Table I). In contrast, dihydrocytochalasin B (diCytB; 20 μM) did not significantly affect the 3H-H2O diffusion coefficient in J774 cells (<5% change, Table I). DiCytB was used as a control for CytB, since it also affects the assembly of actin filaments but does not affect facilitative glucose transport (Mannes and Walsh, 1982).

FIGURE 1. The data in Table I have been used to calculate J774 cell membrane diffusional permeabilities. The legend states the experimental conditions and numbers of experiments.
The permeability coefficients $P_d$ were calculated from the diffusion coefficients using the series-parallel pathway model (Redwood et al., 1974). We utilized one value of $D$ per set of packed cells, and average values for $D_1$ and $D_2$ from parallel experiments. The $P_d$ value for untreated (control) cells was $30.9 \pm 1.8 \mu m/s$. Cells treated with either CytB ($10 \mu M$) or Phl ($20 \mu M$) exhibited lower $P_d$ values ($15.3 \pm 1.9$ and $11.0 \pm 0.7 \mu m/s$, respectively; Fig. 1). These values were significantly different from those in control cells ($t$ test; $P < 0.001$ in both cases). In contrast, J774 cells treated with diCytB ($20 \mu M$) exhibited a $P_d$ value of $28.4 \pm 1.5 \mu m/s$, which is not significantly different from that in untreated cells ($t$ test; $P \sim 0.3$; Fig. 1).

Effect of $p$CMBS on Diffusional Water Permeability and Glucose Transport in J774 Cells

The organomercurial $p$CMBS ($3 \mu M$) reduced $P_d$ by 26% in J774 cells (Fig. 1), and inhibited 2-deoxy-D-glucose uptake into these cells in a dose-dependent fashion (Fig. 2). This reduction in $P_d$ is less than that with either CytB or Phl (Fig. 1), but is significant ($t$ test, $P < 0.01$). As mentioned above, CytB ($10 \mu M$) or $p$CMBS ($3 \mu M$), used singularly, reduced $P_d$ by 50 and 26% respectively (Fig. 1). However, the combination of CytB and $p$CMBS reduced $P_d$ by 87% (Fig. 1).

Effects of $p$CMBS on Osmotic Water Permeability in Oocytes Expressing GLUT1

From the results above, $p$CMBS blocked glucose uptake by $>95\%$ but caused only a 30% reduction in $P_d$ across J774 cells. This disparity led us to examine the effects of $p$CMBS on water permeation through GLUT1 using the oocyte expression system (Vera and Rosen, 1989, 1990; Fischbarg et al., 1990). As previously reported (Fischbarg et al., 1990; Zhang et al., 1991), *Xenopus laevis* oocytes injected with GLUT1 cRNA exhibit a marked increase in osmotic water permeability ($P_l$) (from 13 to 23 $\mu m/s$, Fig. 3 b). However, incubation of both control (water injected) and test (GLUT1 cRNA injected) oocytes with 1 mM $p$CMBS had no effect on $P_l$ (Figs. 3, a and b), indicating that $p$CMBS does not block water movements across GLUT1.
The diffusional movement of water across J774 cells was significantly retarded by CytB and Phi, and not by diCytB (Table I). CytB and Phi also inhibit glucose uptake by J774 cells in a reversible manner, but the analog diCytB does not affect glucose uptake.
uptake (Fischbarg et al., 1989). From the data in Fig. 1, up to 50% of the diffusional permeability to water of the plasma membranes of J774 cells could be mediated by GLUTs.

_Xenopus laevis_ oocytes injected with mRNA encoding GLUT1 exhibit enhanced Pf; this increase in Pf is blocked by Phl (Fischbarg et al., 1990) or CytB (Zhang et al., 1991). These findings led us to propose (Fischbarg et al., 1990; Fischbarg, Kuang, Li, Arant-Hickman, Vera, Silverstein, and Loike, 1993) that GLUTs form channels for transmembrane osmotic water flow. In this light, the most straightforward interpretation of the data presented here is that: (a) GLUTs also behave as channels for transmembrane diffusion of water; (b) glucose and water traverse the same channel through these transporters.

pCMBS (0.5 mM) inhibits glucose uptake through GLUT1 in both oocytes (Wellner, Monden, and Keller, 1992) and J774 cells (Fig. 2) by 80 and 60%, respectively. Both CytB and pCMBS at the concentrations used here (Fischbarg et al., 1989, and Fig. 2, respectively), inhibit glucose uptake by J774 cells by > 95%. Yet, as noted, pCMBS was less effective than either CytB or Phl in inhibiting Pf (Fig. 1). Moreover, pCMBS (1 mM) had no effect on osmotic water permeation in oocytes expressing GLUT1 (Fig. 3, a and b). This raises the question of why pCMBS reduced Pf in J774 cells by some 26% (Fig. 1). In this connection, Zhang et al. (1991) and Preston et al. (1992) have shown that plasma membrane proteins other than GLUTs serve as water channels. The water conductance of CHIP28 is greatly decreased by mercurial compounds (Preston et al., 1992; Zeidel, Ambudkar, Smith, and Agre, 1992a; van Hoek and Verkman, 1992), as is the water conductance of ADH-dependent water channels in urinary epithelia (Ibarra, Ripoche, and Bourguet, 1989). Hence, the additive inhibitory effect of CytB and pCMBS on Pf suggests that pCMBS decreases Pf through effects on water channel proteins other than GLUTs.

Viewed from this perspective, pCMBS binding to sulfhydryl groups on GLUT1 would block glucose transport while sparing the pore through which water traverses GLUTs. In contrast, CytB and Phl block both hexose and water movements across this protein. Hence, whether water passage through a transmembrane protein is affected by sulfhydryl reagents may depend on the location of the sulfhydryl groups relative to the pore.

The Magnitude of the Water Conductance of Facilitative Glucose Transporters

Dempster, van Hoek, de Jong, and van Os (1991), Zeidel, Albalak, Grossman, and Carruthers, 1992b), and Echevarría and Verkman (1992) reported that the same inhibitors of glucose transport that we have used did not measurably reduce Pf in membrane vesicles or cultured cells. Nonetheless, our work (Fischbarg et al., 1989, 1990), and that of Zhang et al. (1991), demonstrated that GLUTs have a finite water conductance (Verkman, 1992). The magnitude of this conductance remains at issue.

We have compared the magnitude of water conductance through GLUTs (Fischbarg et al., 1993) to the water conductance through CHIP28 using estimates of the number of copies of GLUT and of CHIP28 expressed by oocytes injected with cRNA encoding each of these proteins (Fischbarg et al., 1990, and Preston et al., 1992). We have calculated that the GLUT water conductance is ~ 7% of that of CHIP28 (Fischbarg et al., 1993). Therefore, in cells expressing both GLUTs and CHIP28 in
similar amounts (e.g., red blood cells), it will be difficult to detect effects of inhibitors of glucose transport on Pf due to the much larger osmotic conductance of CHIP28. Indeed, it has been reported by several groups that Phl does not measurably affect the osmotic permeability of red blood cells (Macey and Farmer, 1970; Solomon, Chasan, Dix, Lukacovich, Toon, and Verkman, 1983).

Permeability Ratio: Pathways Through Proteins and Lipids

The ratio of Pf (89.6 ± 3.2 μm/s, Fischbarg et al., 1989) to Pd (30.9 ± 1.8 μm/s, Fig. 1) in J774 cells is 2.9 ± 0.1. This is very similar to the 3.1 value obtained by Garrick, Polepka, Cua, and Chinard (1986) for alveolar dog macrophages, suggesting that a substantial fraction of water movement across the plasma membranes of macrophages occurs via transmembrane pores. It has been argued that for most cells (aside from red blood cells and some specialized epithelial cells), their bilayer permeability suffices for the water exchanges necessary for metabolic activity (Finkelstein, 1987). However, in our case, the combination of CytB and pCMBS reduces Pd by 90% (Fig. 1), so 90% of transmembrane water conductance in J774 cells appears to be mediated by membrane proteins. Hence, either macrophages are also specialized cells with high water permeability, or else protein water channels may be more prevalent in cells than has been recognized so far.

A Model for Water Permeation Through GLUTs

We have recently proposed (Fischbarg et al., 1993) a model according to which GLUTs possess a channel with a relatively large diameter (at least 9 Å). Such diameter would confer multifunctional properties to this protein, allowing both water and glucose to traverse it (Fischbarg et al., 1993). In our model, this transporter would possess gates (presumably at the ends of the channel) that fluctuate in position continuously, assuming multiple intermediate conformations between open and closed end states. CytB and Phl could either obstruct the GLUT channel or fix its "gate(s)" in a conformation which would preclude glucose and water movements. In contrast, pCMBS could fix either the gates or the protein channel in a partially open position, so that water (1.4 Å in radius) but not glucose (~4.0 Å in radius) could traverse the channel. Alternatively, pCMBS may bind to the exofacial domain of GLUT1, thereby restricting access of glucose but not of water to the channel's entrance.

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