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Glucose transporters serve as water channels

(water osmotic permeability/*Xenopus laevis* oocytes/mRNA expression/water pore)

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ABSTRACT **Water traverses the plasma membranes of some eukaryotic cells faster than can be explained by the water permeability of their lipid bilayers. This has led to a search for a water channel. Our previous work identified glucose transporters as candidates for such a channel. We report here that *Xenopus laevis* oocytes injected with mRNA encoding the brain/Hep G2, adult skeletal muscle/adipocyte, or liver forms of the glucose transporter exhibit an osmotic water permeability of their plasma membranes larger than that of untreated oocytes. The osmotic water permeability component attributable to glucose transporters increased an average of 4.8-fold in the injected oocytes. These studies provide direct evidence that the facilitative, sodium-independent mammalian glucose transporters serve as membrane water channels.**

Virtually all mammalian cells express proteins that mediate the stereospecific transport of D-glucose across their plasma membranes by facilitated diffusion (1). This mode of transport is characteristic of glucose transporters (GTs), which have been molecularly identified in brain (2, 3), skeletal muscle and adipocytes (4–7), hepatocytes (8, 9), and fetal muscle (10). In addition, several investigators have suggested, on the basis of experimental (11, 12) and theoretical (13) considerations, that the GT may contain a water-filled channel that spans the membrane.

We have provided evidence that in the macrophage-like J774 cell line, GTs serve as water channels (14). Our evidence (14) was based on the observation that inhibitors of glucose transport significantly reduce the rate of osmotic water flow across the cell's plasma membrane. In those studies, the osmotic water permeability (*P*) was monitored by measuring the rate of cell volume change in response to an osmotic challenge. In cells exposed to either hypotonic or hypertonic challenge in the presence of a specific inhibitor of glucose transport, cytochalasin B, *P* was reduced from 85 μm/sec to 25 μm/sec. The latter value is consistent with the *P* of lipid bilayers (15).

To rigorously test the hypothesis that GTs serve as water channels, we have expressed mammalian GTs in frog oocytes as described by Vera and Rosen (16) and have compared the *P* value of these oocytes with that of control oocytes. Specifically, oocytes injected with mRNA encoding either brain/Hep G2 GT (GT2), adult skeletal muscle/adipocyte GT (GT1), or liver GT (GT3) exhibit a 20- to 40-fold increase in the rates of uptake of 2-deoxy-D-glucose or 3-*O*-methylglucose above those values observed in control or uninjected oocytes (16, 17). In addition, the uptake of 2-deoxy-D-glucose in mRNA-injected oocytes was inhibited by cytochalasin B (16), by phloretin (Phl; data not shown), and by elevated concentrations of D-glucose, but not by L-glucose (16). Thus, mammalian GTs expressed in *Xenopus* oocytes retain characteristic properties of GTs from mammalian cells.

Given this background, we injected *Xenopus laevis* oocytes with mRNA encoding three different GTs originally cloned from rat brain, adult skeletal muscle, and liver cDNA libraries (2–9) and have measured the rate of swelling of the oocytes in response to hypoosmotic challenge. We report here that in oocytes expressing any one of these transporters, the *P* value increased significantly compared with values observed in control oocytes (water-injected or uninjected). These results directly show that GTs serve as water channels.

METHODS

Isolation of Oocytes and mRNA Injection. Ovaries were removed from mature *Xenopus laevis* as described (16) and suspended in Barth's solution (18). Oocytes were dissociated from the ovary, and follicle cells were removed. RNA was dissolved in distilled water at 0.2 to 0.4 mg/ml, and 50 nl was injected into the cytoplasm of stage VI oocytes as described; some control oocytes were injected instead with 50 nl of water. Oocytes were then placed in tissue culture dishes in Barth's medium for 2–3 days at 18°C.

Oocyte Volume Measurements and Osmotic Water Permeability Determinations. To conduct an experiment, an oocyte was placed in a well of a 96-well tissue culture dish filled with Barth's medium and was allowed to equilibrate for 20 min at room temperature, during which time its volume was monitored (see below) to ensure that it remained constant. At that point, the bathing solution was aspirated and replaced with 50% (vol/vol) hypotonic Barth's solution, and the oocyte diameters were measured at predetermined time intervals. All volume measurements were conducted at room temperature. Oocyte size was determined by using an inverted microscope fitted with a ×10 objective lens, a ×4.2 ocular lens, and a video camera. The image obtained was viewed on a video monitor. Oocyte diameter was measured on the video screen at two axes perpendicular to each other, and oocyte volume at each time point was calculated for an ellipsoid by using the measured diameters. After each osmotic challenge, diameters were measured every 2 min for the first 20 min, every 5 min for the next 20 min, and every 10 min thereafter. The data presented in Figs. 1 and 2 describing normalized oocyte volumes as a function of time were analyzed with an algorithm (14) to calculate *P* values and unstirred layer thickness (*d*) values.

RESULTS

P in oocytes was measured by monitoring the volume of the oocytes before and after a change in the tonicity of ambient

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Abbreviations: *P*, osmotic water permeability; *d*, unstirred layer thickness; Phl, phloretin; GT, glucose transporter.

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Barth's medium (18). A 50% hypotonic medium achieved by lowering the NaCl molar concentration had a tonicity 0.5 times that of Barth's medium. In Barth's (isotonic) medium, control oocytes (n = 9) and mRNA-injected oocytes (n = 21) had volumes of 1.13 ± 0.03 and 1.17 ± 0.07 mm^3 (mean ± SEM), respectively. Oocytes exposed to hypotonic solution slowly increased their volume in an exponential-like fashion (Fig. 1). A 20% increase in cell volume was achieved within 42 min after osmotic challenge, and a steady-state volume about 30% larger than the original one was observed at 2 hr. This is consistent with prior reports (19) showing that untreated oocytes exhibit a relatively low P value and that a large part of their volume is excluded from osmotic exchange. We did not observe any volume regulation in oocytes exposed to hypotonic solutions and observed only a very slow (over several hours) reduction in oocyte volume in response to hypertonic solutions (data not shown).

Oocytes were tested for their P value 2–3 days after injection with 10–25 ng of mRNA encoding the brain-, muscle-, or liver-type GTs. On the average, oocytes expressing any one of the GTs exhibited a much more rapid response to osmotic challenge than did water-injected or uninjected oocytes. For example, oocytes injected with brain-type GT mRNA responded to a 50% hypotonic challenge in an exponential-like fashion, such that a 20% increase in cell volume occurred within 27 min (Fig. 1). There was no significant difference in the kinetics of osmotic response to 50% hypotonic challenge between oocytes expressing the brain-, muscle-, or liver-type GTs. However, oocytes injected with either mRNA or H_2O were noted to be fragile and sometimes ruptured at the injection site 25–60 min after hypoosmotic challenge. Therefore, we terminated the collection of data for most injected oocytes before they reached osmotic equilibrium (Fig. 1).

To determine whether inhibitors of glucose-facilitated diffusion also slow water movement across oocyte membranes [as they do across macrophage membranes (14)], we treated the oocytes with Phl. Oocytes expressing brain-type GTs were preincubated for 30 min in isotonic Barth's medium containing 20 μM Phl and then were challenged with a 50% hypotonic solution also containing 20 μM Phl. Compared with similarly injected oocytes to which no inhibitor was added, these oocytes exhibited a much decreased rate of volume change; in fact, their rate of volume change was even slightly slower than that observed in noninjected (or water-injected) oocytes (Fig. 2). Water-injected oocytes treated with 20 μM Phl exhibited P values similar to those of oocytes injected with brain-type GT mRNA and also treated with Phl (Fig. 2). Note that Phl slightly decreases P [as well as the glucose uptake (data not shown)] of water-injected oocytes. We believe this is due to inhibition of endogenous oocyte GTs (17); the observation that the P value of Phl-treated oocytes expressing brain GTs is similar to that observed in Phl-treated water-injected oocytes is consistent with this explanation. Thus, Phl inhibits the component of P directly attributable to the presence of brain-type GTs. Control experiments demonstrated that Phl completely blocked 2-deoxy-D-glucose uptake in oocytes injected with brain-type GTs (data not shown). These results are consistent with our previous report that Phl markedly reduces the P value of J774 cells (14) by blocking their GTs.

To examine whether expression *per se* of novel membrane proteins affects P, oocytes were injected with mRNA encoding a form of the human insulin receptor that is mutated in its ATP-binding domain (Lys-1018 to Ala-1018). The externally disposed domain of this mutant receptor retains insulin-binding activity, but the protein kinase on the receptor's cytoplasmic domain is inactive (17). This mutated receptor was chosen as a control protein because it is a membrane-spanning glycoprotein that does not function as a transporter. From the amount of insulin that bound to control oocytes and to oocytes expressing this mutated insulin receptor, we calculate that control oocytes express 10^5 insulin receptors and that oocytes injected with mRNA encoding insulin receptors express 10^9 receptors per oocyte (17). Despite this marked increase in membrane-spanning proteins, oocytes expressing mutated insulin receptors responded to osmotic challenge similarly to control oocytes (Fig. 2). These results show that the presence of a novel integral membrane protein in the oocytes' plasma membrane does not alter the rate of

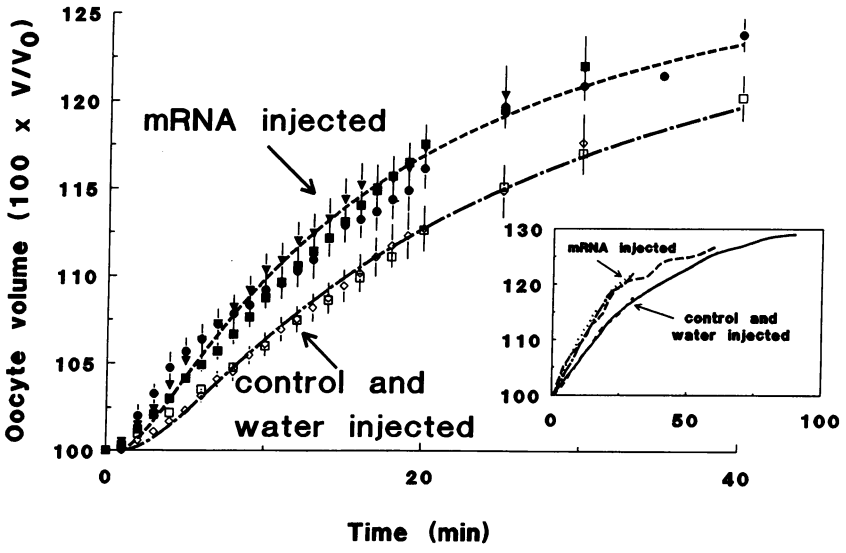

FIG. 1. Effect of expression of rat GTs in frog oocytes on their P value. Oocyte volume was calculated from the measured diameters (see text) and was expressed as the relative volume V/V_0 (V = volume at a given time; V_0 = initial volume) multiplied by 100. Average (± SEM) relative volumes are plotted as a function of time; each data set represents measurements from five to eight oocytes. The time interval shown here is limited to the initial 40 min. Control oocytes were either untreated (□) or injected with water (◇); mRNA-injected oocytes were injected with 10–25 ng of the mRNA encoding the brain (●), muscle/adipocyte (▼), or liver (■) GTs from rat. Curves were fitted to the averaged data by using an algorithm (14) to calculate P and d; the values utilized for the upper curve were P = 81 μm/sec and d = 615 μm and for the lower curve were P = 53.5 μm/sec and d = 832 μm. (*Inset*) The complete sets of data are shown here. Lines connect the average values for each set.

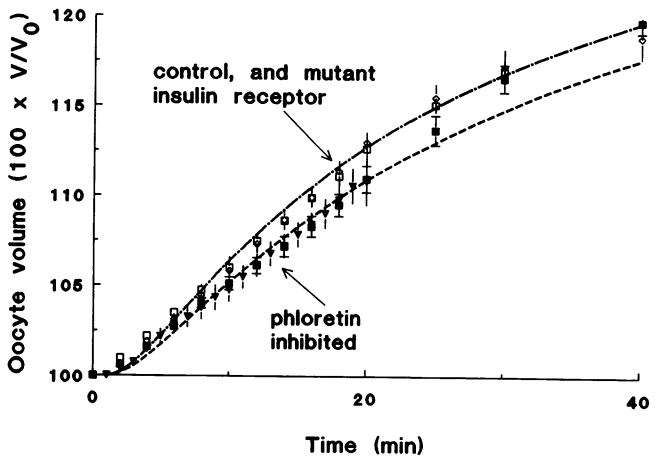

FIG. 2. Phl prevents an increase in P value in oocytes expressing brain GTs. Experiments were performed as described in the legend of Fig. 1 and in the text. Water-injected oocytes (■) or oocytes expressing brain GTs (▼) were preincubated in Barth's medium containing 20 μM Phl for 30 min, at which point the bathing solution was aspirated and replaced with 50% hypotonic Barth's medium also containing 20 μM Phl. In additional control experiments, P was measured in oocytes expressing a mutant human insulin receptor (◇) (see text). For comparison, the data for control oocytes from Fig. 1 (□) are presented here also. Average (± SEM) relative volumes are plotted as a function of time; each data set represents measurements from five to seven oocytes. Curves were fitted as in Fig. 1; the values for the lower curve were P = 45.6 μm/sec and d = 822 μm.

osmotically driven water flux into these cells. They support our contention that GTs function as water channels.

It is of interest that oocytes injected with mRNA for the native (kinase replete) insulin receptor exhibited slightly higher constitutive (i.e., in the absence of insulin) levels of P (data not shown) and glucose uptake (17) than uninjected oocytes or oocytes expressing kinase-negative receptors. We interpret this to mean that even in the absence of insulin, the kinase-replete receptor has a small but measurable effect on the number of GTs expressed by oocytes.

DISCUSSION

How might the GT facilitate water movement across the plasma membrane? The model of Mueckler *et al.* (2) of the tertiary structure of the Hep G2/brain-type GT predicts the presence of 12 membrane-spanning helices. Since several of these helices are amphipathic, Mueckler *et al.* (2) have suggested that their abundant hydroxyl and amide side chains line a transmembrane pore through which the sugar moves. Presumably glucose binds to these side chains as it transits the pore. Similarly, water movement through this channel may also involve hydrogen bonding to such sites and saltatory movements between sites.

Our calculation of the rate of water movement across each GT is consistent with the idea that water transits a pore in the transporter molecule. Assuming oocytes have an ellipsoidal shape and a smooth surface, we calculate their average volume and surface area to be 1.14 ± 0.02 mm^3 and 5.3 ± 0.1 mm^2, respectively. Using a curve-fitting procedure (14) for the data in Fig. 1, we calculate osmotic permeabilities of control (P_c) oocytes and of oocytes injected with brain GT mRNA (P_{inj}) to be 53.5 and 83.5 μm/sec, respectively. However, studies of toad oocytes (20) show that these cells have numerous villus processes, which increase the area of their surface membrane 9-fold above the value calculated for a smooth-surface ellipsoid of comparable size. Assuming *Xenopus* oocytes have similar villi, we calculate their surface area (A_c) to be 48 mm^2. Using this value, we calculate osmotic permeabilities of control (P_c) oocytes and of oocytes injected with brain GT mRNA (P_{inj}) to be 5.9 and 9.3 μm/sec, respectively. This P value for control oocytes is larger than a value of 2.9 μm/sec previously reported (20) for the P value of toad oocytes of the same size as those presently used (1.3-mm average diameter). In contrast to our calculations, the determination for the P value of toad oocytes did not correct for the large unstirred layers existing inside and outside the oocyte. In our system, the unstirred layer values were between 600 and 800 μm/sec for both control and injected oocytes.

To determine the net P value attributable to the GTs, the average P value calculated for Phl-treated uninjected and injected oocytes (45.7 μm/sec) was subtracted from the average P values for control oocytes (53.5 μm/sec) and GT2 mRNA-injected oocytes (83.5 μm/sec). In control oocytes, the net P value (attributable to the GT) was 53.5–45.7 = 7.8 μm/sec. In GT2 mRNA-injected oocytes, the net P value was 83.5–45.7 = 37.8 μm/sec. Thus, the net P value of injected oocytes was almost 5-fold greater than that of control ones. This 5-fold increase is less than expected when compared with the 30-fold average increase we have measured (ref. 16 and data not shown) in V_{max} values for glucose uptake in brain mRNA-injected oocytes versus control ones. We attribute this disparity to the fact that the osmotically induced water flow into oocyte microvilli will tend to dilute the intravillar salt concentration, thereby reducing the osmotic gradient and water movement across microvillar membranes.

V_{max} values for glucose uptake into control and brain GT mRNA-injected oocytes were 2.0 and 57.5 pmol/min per oocyte (data not shown). At the saturated zero trans conditions of our measurements, the turnover value for the rat erythrocyte GT has been reported to be 16 sec^{-1} (21). Using that turnover value, we calculate that control oocytes (n_c) and mRNA-injected oocytes (n_{inj}) express 1.2 × 10^9 and 3.6 × 10^{10} transporters per oocyte, respectively. The rate of turnover for osmotic transport of water molecules per transporter (rtw) can be calculated (14) from the equation: rtw = $(P_{inj}-P_c)\,\Delta\pi\,A_c\,N/(n_{inj}-n_c)$, where the osmotic gradient = $\Delta\pi$ = 0.080 mol/liter, N = Avogadro's number, and all other values are as defined above. This yields rtw = 2.8 × 10^5 molecules per sec per transporter. Assuming further that many of the GTs in the microvilli are transporting water at suboptimal rates because of the dilutional effects discussed above, we correct rtw by multiplying it by the fractional increase in glucose uptake (28.8) over the fractional increase in P attributable to GTs (4.8). With this correction, rtw becomes 1.7 × 10^6 molecules per sec per transporter. Both the raw and corrected turnover numbers (2.8 × 10^5 and 1.7 × 10^6, respectively) are within the range expected from theoretical considerations (13) for water molecules traversing at 4.5-Å radius pore, such as may exist across a GT. These rtw values are less by a factor of 5–50 than the calculated rate for unidirectional sodium ion flux through a gramicidin A pore (22) and 10^3–10^5 greater than the estimated turnover rates for glucose movement through its transporter (23).

In both oocytes (this paper) and J774 cells (14), water movement across the transporter occurs in the absence of glucose in the extracellular medium. Hence, glucose is not needed to "open" the water channel, suggesting that the putative hydrophilic pore of the transporter is inherently accessible to water.

If the GT is always "open" to water, what prevents small ions from traversing such pores and dramatically altering cellular ionic concentrations? We speculate that the transporter may present an electrostatic filter against such ions. The published amino acid sequence for the Hep G2/brain-type GT (2) shows the presence of many positively and negatively charged residues at the putative extracellular segment between helices 1 and 2, at the intracellular seg-

ments between helices 6 and 7, between helix 12 and the carboxyl end of the protein, and in the shorter connecting loops between the other helices. The ensembles of charged residues may act as electrostatic filters on both sides of the transporter.

In conclusion, the results reported here demonstrate that the facilitative, sodium-independent mammalian GT, when expressed in *Xenopus laevis* oocytes, serves as a membrane water channel. Furthermore, three distinct but homologous forms of GT, each expressed in different cell types, serve equally well to facilitate water flow into the oocyte. Thus, facilitative GTs also must function as water channels in each cell that expresses them. We hypothesize that the wide range of osmotic permeabilities measured in different types of mammalian cells may be related to the number of membrane GTs expressed by each of these cells. Although it is not yet known whether other transporters or ion channels serve as water channels, these studies identify GTs as leading candidates for the heretofore elusive cell membrane water channel.

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