Nicotinamide Is Not a Substrate of the Facilitative Hexose Transporter GLUT1 †

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Nicotinamide Is Not a Substrate of the Facilitative Hexose Transporter GLUT1†

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ABSTRACT: It has been proposed that GLUT1, a membrane protein that transports hexoses and the oxidized form of vitamin C, dehydroascorbic acid, is also a transporter of nicotinamide (Sofue, M., Yoshimura, Y., Nishida, M., and Kawada, J. (1992) *Biochem. J. 288*, 669−674). To ascertain this, we studied the transport of 2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, and nicotinamide in human erythrocytes and right-side-out and inside-out erythrocyte membrane vesicles. The transport of nicotinamide was saturable, with a K_M for influx and efflux of 6.1 and 6.2 mM, respectively. We found that transport of the hexoses was not competed by nicotinamide in both the erythrocytes and the erythrocyte vesicles. Likewise, the transport of nicotinamide was not affected by hexoses or by inhibitors of glucose transport such as cytochalasin B, genistein, and myricetin. On the other hand, nicotinamide blocked the binding of cytochalasin B to human erythrocyte membranes but did so in a noncompetitive manner. Using GLUT1-transfected CHO cells, we demonstrated that increased expression of GLUT1 was paralleled by a corresponding increase in hexose transport but that there were no changes in nicotinamide transport. Moreover, nicotinamide failed to affect the transport of hexoses in both control and GLUT1-transfected CHO cells. Therefore, our results indicates that GLUT1 does not transport nicotinamide, and we propose instead the existence of other systems for the translocation of nicotinamide across cell membranes.

Mammalian cells possess highly specialized systems for the transport of nutrients and other solutes across the plasma membrane. The facilitative hexose transporters (GLUTs) are a family of integral membrane proteins that transport hexoses down a concentration gradient and are expressed in all cells and tissues (*1*, *2*). Twelve genes encoding different hexose transporter isoforms have been molecularly cloned (*3*−*14*). The various members within the family of facilitative hexose transporters differ in primary structure and functional properties (*15*). GLUT1 is a high affinity glucose transporter that is expressed in all cells and is especially abundant in erythrocytes and brain (*3*, *16*). GLUT2 is present in liver, small intestine, and kidney and is a low affinity glucose transporter that also transports fructose but with an even lower affinity (*5*, *17*). GLUT3 is a high affinity glucose transporter present in neuronal cells (*6*). GLUT4 is expressed in adipose tissue and muscle and is responsible for the acute increase in glucose utilization after insulin stimulation (*8*). GLUT5 is not a glucose transporter but instead is an efficient fructose transporter that is expressed in cells such as intestinal enterocytes and spermatozoa (*18*−*20*). Less information is available about the specificity and properties of the recently cloned isoforms (*12*−*15*, *21*, *22*).

The glucose transporters GLUT1, GLUT2, GLUT3, and GLUT4 are also efficient transporters of the oxidized form of vitamin C, dehydroascorbic acid (*23*, *24*). In addition, glucose transporters are permeable to water (*25*, *26*), and it is also known that GLUT1 interacts with compounds that have no obvious structural similarities to glucose or dehydroascorbic acid. These compounds, which are not transported by GLUT1, include the alkaloid cytochalasin B, the chalcone phloretin, steroids, benzoic acid derivatives, and barbiturates (*1*, *27*−*29*). We recently showed that a group of tyrosine kinase inhibitors, which include natural products of the family of flavones and isoflavones and synthetic compounds such as tyrphostins inhibit the functional activity of GLUT1 by directly interacting with the transporter (*30*).

Against this background, the report that GLUT1 transported nicotinamide in a reconstituted system (*31*) implied that perhaps the selectivity of GLUT1 for hexoses was less strict than was commonly accepted. However, in an attempt to correlate this finding with the physiological cell environment, we noted that the kinetic constants associated with the uptake of nicotinamide in mammalian cells differ by at least 1 order of magnitude from those calculated in the reconstituted system (*32*). This is in contrast with results from

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reconstitution experiments which have demonstrated that the kinetic characteristics for the transport of hexoses by GLUT1 reconstituted in vesicles are very similar to those determined in whole cells. Therefore, we studied in detail the interaction between nicotinamide and hexose transport in human erythrocytes and in resealed right-side-out and inside-out human erythrocyte ghosts. We found that the transport of hexoses was not competed by nicotinamide and that nicotinamide transport was not affected by hexoses or by inhibitors of glucose transport. On the other hand, nicotinamide did block the binding of cytochalasin B to human erythrocyte membranes in a noncompetitive manner. Our results suggest that GLUT1 does not transport nicotinamide.

MATERIALS AND METHODS

Human erythrocytes were purified from human blood samples provided by the Blood Bank Service of the Regional Hospital in Valdivia. Chinese hamster ovary (CHO)[1] cells expressing GLUT1 (*33*) were cultured in IMDM supplemented with 10% fetal bovine serum and 0.25 mg/mL G418. Right-side-out and inside-out vesicles were prepared from human erythrocytes as previously described (*34*). The fraction of vesicles oriented inside-out was estimated by assaying cholinesterase activity in the absence or the presence of Triton X-100. Approximately 90% of the vesicles were oriented inside-out.

For uptake assays, erythrocytes were incubated at room temperature in incubation buffer containing 0.1−1 μCi L-[^{14}C-carbonyl]nicotinamide (specific activity, 35 mCi/mmol, Sigma Chemical Co.), 2-[1,2-^{3}H(N)]deoxy-D-glucose (26.2 Ci/mmol, NEN-DuPont), or [^{3}H]-3-*O*-methy-D-glucose (86.7 Ci/mmol, NEN-DuPont, or 60 Ci/mmol, ARC). The final substrate concentrations were adjusted adding the respective unlabeled compounds. The incubation times were varied from 5 s to 3 min as indicated in the respective figure legends, and uptake was stopped by adding 10 volumes of cold phosphate-buffered saline (4 °C). The cells were collected and washed twice by centrifugation into cold phosphate-buffered saline that was free of Ca^{2+} and Mg^{2+} and subsequently lysed in methanol. The incorporated radioactivity was determined by liquid scintillation counting. When testing the effects of different competitors and inhibitors on uptake, they were added at the beginning of the experiment from freshly prepared concentrated stock solutions.

For right-side-out and inside-out vesicles, uptake assays were performed at room temperature as described by Jarvis (*35*) in a final volume of 200 μL of a vesicle suspension in 5 mM Hepes (pH 8.0) containing 400−800 μg of protein. A 1−1.2 μCi aliquot of labeled substrate and enough cold substrate to attain the concentrations shown in the figures were added with vigorous agitation at time zero. Uptake was stopped by adding 5 mL of cold stopping solution (100 μM phloretin, 2 μM $HgCl_2$, 1.5 mM KI, and 5 mM Hepes (pH 8.0)), and the suspension was filtered through Millipore filters (0.45 μm pore size). The filters were then washed 3 times with 5 mL of cold stopping solution, and the radioactivity retained in the filters was determined by liquid scintillation counting.

Transport assays using CHO cells were performed as described for erythrocytes, except that, after uptake and washing, the cells were solubilized in 10 mM Tris-HCl (pH 8.0) containing 0.2% sodium dodecyl sulfate (SDS) and processed for liquid scintillation counting (*33*). The results shown in all uptake assays represent the average of four independent experiments.

Pink erythrocyte ghosts were prepared from washed red cells as previously described (*34*). D-Glucose inhibitable binding of cytochalasin B to functional glucose carriers was estimated from the difference between cytochalasin B bound in the presence of 500 mM L-glucose (or D-sorbitol) and 500 mM D-glucose. Binding assays were performed in a final volume of 150 μL, containing 0.06−0.1 mg/mL erythrocyte membrane protein (equivalent to 1−1.6 × 10^8 cells), 10 μM cytochalasin E, 500 mM D- or L-glucose (D-sorbitol), 0.05 μCi [4-^{3}H]cytochalasin B (11.9 Ci/mmol, NEN-DuPont) and cold cytochalasin B for final concentrations of 0.01−5 μM. After 10 min at room temperature, the membranes were collected by centrifugation at 15 000*g* for 10 min. The amount of specifically bound cytochalasin B was estimated by determining the quantity of radioactive ligand associated with the membrane pellet (*33*).

RESULTS

Transport of Hexoses and Nicotinamide by Human Erythrocytes. In initial experiments, we studied the time course of 2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, and nicotinamide uptake in human erythrocytes (Figure 1, parts A and B), and afterward we determined the kinetic properties of nicotinamide uptake (Figure 1, parts C and D). The time course experiments revealed that the uptake of 2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, and nicotinamide occurred rapidly, with half of the maximum concentration being reached in about 20 s, and the system reached steady-state at 60 s or less (Figure 1, parts A and B). Dose−response experiments indicated that the uptake of nicotinamide reached saturation at millimolar concentrations (Figure 1C), with an apparent K_M for transport of 5.0 mM (Figure 1D). Similar dose−response experiments showed that the uptake of both 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose were saturable, with apparent K_M values of 2.5 and 5.5 mM, respectively (data not shown).

We next analyzed the interactions between 2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, and nicotinamide and their respective uptake mechanisms (Figure 1, parts E and G). The uptake of 2-deoxy-D-glucose was competed in a dose-dependent manner by the GLUT1 substrates D-glucose and 3-*O*-methyl-D-glucose (Figure 1E). On the other hand, neither L-glucose, a hexose unable to interact with GLUT1, nor nicotinamide had any discernible effect on 2-deoxy-D-glucose uptake by the erythrocytes (Figure 1E). Similarly, the uptake of 3-*O*-methyl-D-glucose was competed by D-glucose and 3-*O*-methyl-D-glucose but not by L-glucose or nicotinamide (Figure 1F). Confirming the lack of cross-interactions between the hexose and nicotinamide transport systems in the human erythrocyte, nicotinamide uptake was not competed by D-glucose, 2-deoxy-D-glucose, or 3-*O*-methyl-D-glucose (Figure 1G), each at concentrations that produced a total inhibition of their uptake. As expected, L-glucose failed to affect the uptake of nicotinamide by the human erythrocytes.

[1] Abbreviations: OMG, 3-*O*-methyl-D-glucose; DOG, 2-deoxy-D-glucose; NAM, nicotinamide; L-Glc, L-glucose; D-Glc, D-glucose; Cyt B, cytochalasin B.; CHO, Chinese hamster ovary.

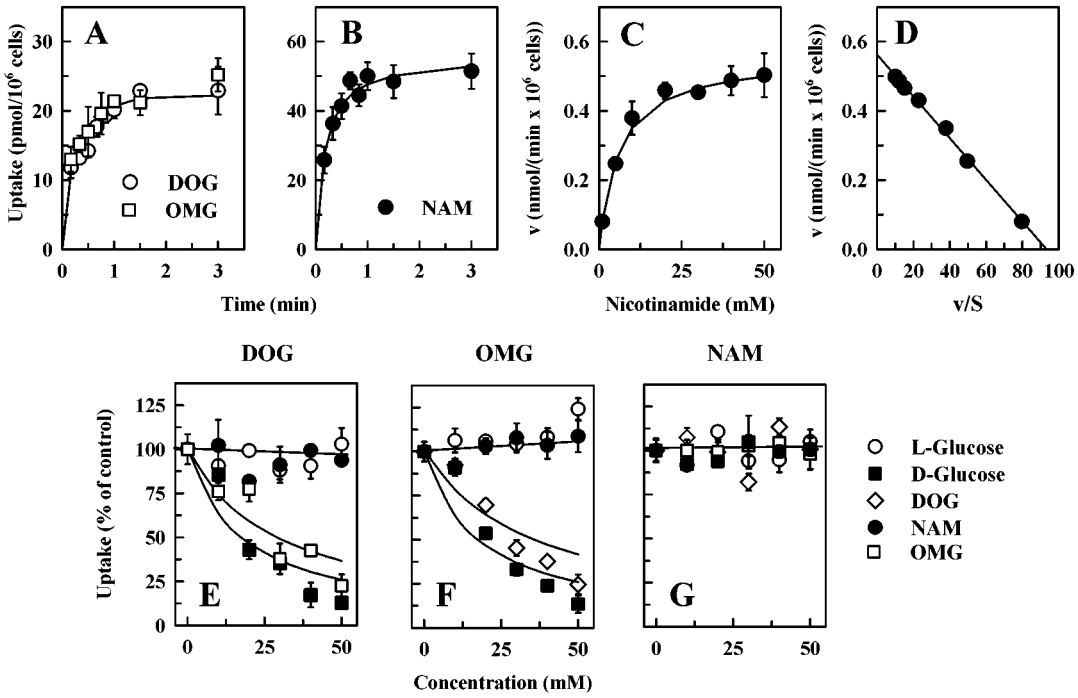

FIGURE 1: Uptake of 2-deoxy-D-glucose (DOG), 3-*O*-methyl-D-glucose (OMG), and nicotinamide (NAM) into human erythrocytes. (A) Time course of the incorporation of (○) 0.5 mM DOG or (□) 0.5 mM OMG. (B) Time course of 1 mM NAM incorporation. (C, D) Kinetics of nicotinamide uptake by human erythrocytes. (C) Saturation curve. (D) Plot of v against v/S. (E, F) Uptake of 0.5 mM (E) DOG or (F) OMG in the presence of increasing nicotinamide and hexose concentrations. (G) Uptake of 1 mM NAM in the presence of increasing hexose concentrations. Assays were done at room temperature using an average number of 4.3×10^7 cells per assay.

Transport of Hexoses and Nicotinamide by Right-Side-Out Erythrocyte Vesicles. To confirm these results and to rule out possible confounding effects of intracellular components, we repeated the uptake and competition experiments using right-side-out erythrocyte membrane vesicles. In general terms, the results were similar to those obtained with the intact erythrocytes: (1) the uptake of 2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, and nicotinamide occurred rapidly (Figure 2A), with half of the maximum concentration reached in less than 20 s and maximum incorporation at about 60 s; (2) the uptake of nicotinamide was saturable (Figure 2B), with an apparent K_M of 6.1 mM (Figure 2C); (3) the uptake of 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose was competed by the hexoses that are GLUT1 substrates but not by L-glucose (Figure 2, parts D and E); and (4) nicotinamide failed to affect the uptake of 2-deoxy-D-glucose (Figure 2D) and 3-*O*-methyl-D-glucose (Figure 2E) and vice versa, 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose failed to affect the uptake of nicotinamide (Figure 2F).

Uptake of Hexoses and Nicotinamide by Inside-Out Erythrocyte Vesicles. Taken together, these data indicate that interactions between nicotinamide and the exofacial side of the transporter are unlikely. However, because GLUT1 transports hexoses bidirectionally down a concentration gradient, we investigated whether nicotinamide could interact with the endofacial end of the transporter. To ascertain this issue, we studied the uptake of hexoses and nicotinamide by inside-out erythrocyte membrane vesicles. The results of this series confirmed that while both nicotinamide and hexoses are transported by the inside-out vesicles, nicotinamide did not affect the uptake of the hexoses and vice versa (Figure 3). The uptake of 2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, and nicotinamide occurred rapidly (Figure 3A), with half of the maximum concentration reached in less than 20 s and maximum incorporation in about 60 s. The uptake of nicotinamide was saturable (Figure 3B), with an apparent K_M of 6.2 mM (Figure 3C). The uptake of 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose was competed by the hexoses that are GLUT1 substrates but not by L-glucose (Figure 3, parts D and E). Nicotinamide failed to affect the uptake of 2-deoxy-D-glucose (Figure 3D) and 3-*O*-methyl-D-glucose (Figure 3E) and vice versa, 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose failed to affect the uptake of nicotinamide (Figure 3F).

Effect of Cytochalasin B and Other GLUT1 Inhibitors. Sofue et al. reported that nicotinamide displaced cytochalasin B from erythrocyte membranes. We confirmed this finding; increasing concentrations of nicotinamide efficiently competed for the D-glucose-sensitive cytochalasin B binding sites present in the erythrocyte membranes (Figure 4A). Approximately 30 mM nicotinamide inhibited the binding of 0.1 μM cytochalasin B by 50%, while total inhibition of binding was observed at 100 mM nicotinamide. As expected, L-glucose had no effect on cytochalasin B binding (Figure 4A), while approximately 50 mM D-glucose inhibited the binding of cytochalasin B by 50%. Further analysis revealed that nicotinamide displaced cytochalasin B in a noncompetitive manner (Figure 4B). Nicotinamide was a linear noncompetitive blocker of cytochalasin B binding to the erythrocyte ghosts, because a secondary plot of the apparent extent of cytochalasin B binding as a function of nicotinamide concentration was linear and gave a noncompetitive inhibition constant for nicotinamide of approximately 11 mM (Figure 4C). Moreover, cytochalasin B failed to affect the uptake of nicotinamide by human erythrocytes (Figure 4D), even at concentrations that completely blocked the uptake

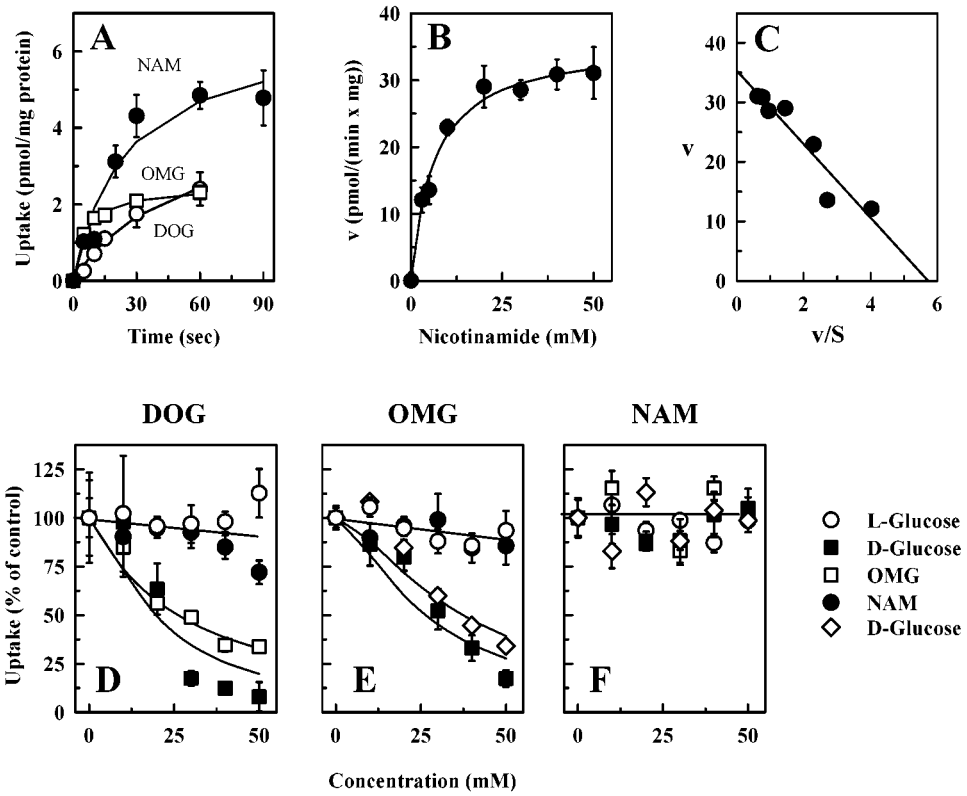

FIGURE 2: Uptake of 2-deoxy-D-deoxyglucose (DOG), 3-*O*-methyl-D-glucose (OMG), and nicotinamide (NAM) into human erythrocytes right-side-out sealed vesicles. (A) Time course of uptake of (○) 0.5 mM DOG, (□) 0.5 mM OMG, and (●) 1 mM NAM. (B, C) Kinetics of nicotinamide uptake. (B) Saturation curve. (C) Plot of v against v/S. (D, F) Effect of hexoses and nicotinamide on the uptake of (D) 0.5 mM DOG, (E) 0.5 mM OMG, and (F) 1 mM NAM. Uptakes were performed at room temperature using 5 s uptake assays.

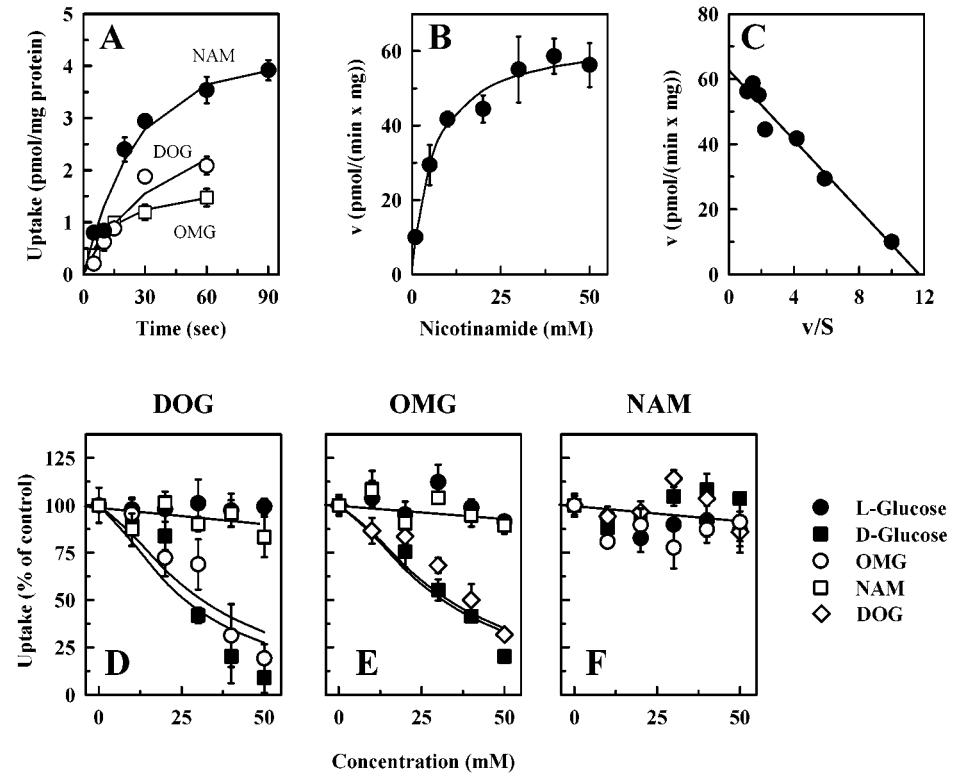

FIGURE 3: Uptake of 2-deoxy-D-glucose (DOG), 3-*O*-methyl-D-glucose (OMG), and nicotinamide (NAM) into human erythrocytes inside-out sealed vesicles. (A) Time course of uptake of (○) 0.5 mM DOG, (□) 0.5 mM OMG, and (●) 1 mM NAM. (B, C) Kinetics of nicotinamide uptake. (B) Saturation curve. (C) Plot of v against v/S. (D, F) Effect of hexoses and nicotinamide on the uptake of (D) 0.5 mM DOG, (E) 0.5 mM OMG, and (F) 1 mM NAM. Uptakes were performed at room temperature using 5 s uptake assays.

of 3-*O*-methyl-D-glucose (Figure 4E). The same behavior was observed using other glucose transporter inhibitors such as the isoflavone genistein and the flavone myricetin, which potently inhibited the GLUT1-mediated transport of meth-

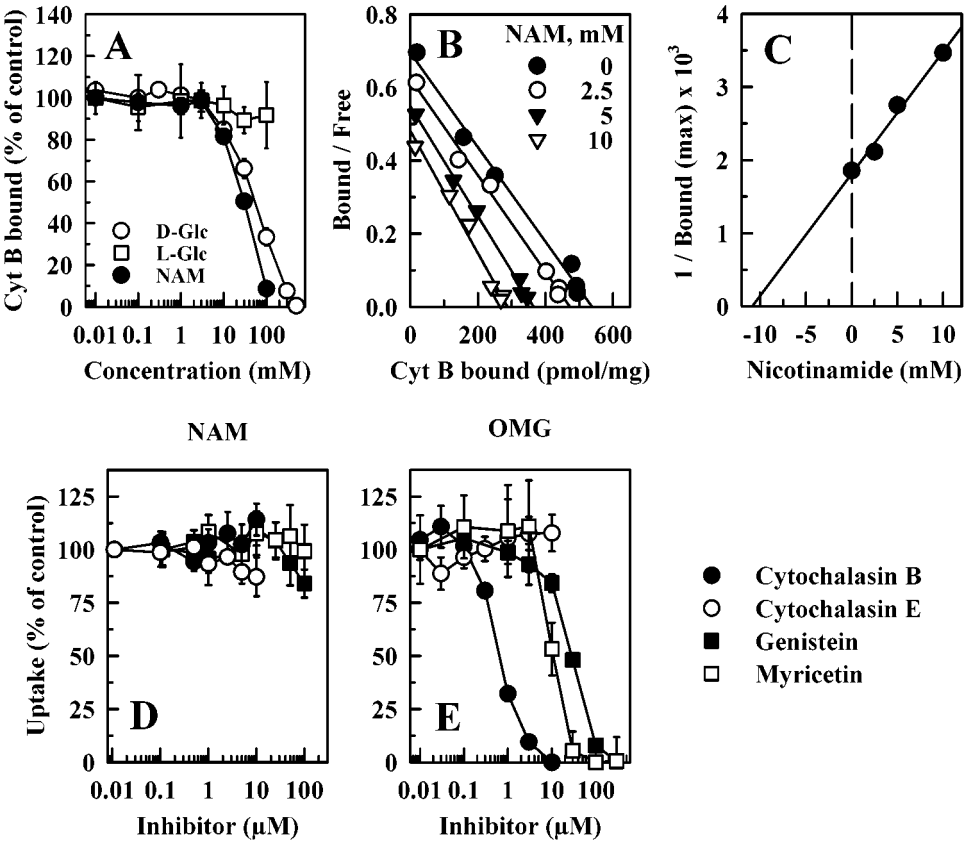

FIGURE 4: Effect of nicotinamide (NAM) and glucose transporter inhibitors on the binding of cytochalasin B and the uptake of nicotinamide and 2-deoxy-D-glucose (DOG) in human erythrocytes. (A−C) Effect of nicotinamide on the binding of cytochalasin B to human erythrocyte membranes. (A) Binding of cytochalasin B in the presence of increasing concentrations of (□) L-glucose, (○) D-glucose, or (●) NAM. (B) Scatchard plot of the effect of nicotinamide at (●) 0, (○) 2.5, (▼) 5, and (▽) 10 mM on the binding of increasing cytochalasin B concentrations. (C) Secondary plot of the nicotinamide effect on the glucose-sensitive cytochalasin B binding to the erythrocyte membranes. Incubations were performed at room temperature using 20-min assays. (D, E) Effect of glucose transporter inhibitors on the uptake of nicotinamide and 2-deoxy-D-glucose by human erythrocytes. (D) Uptake of 1 mM nicotinamide in the presence of increasing concentrations of (●) cytochalasin B, (○) cytochalasin E, (■) genistein, and (□) myricetin. (E) Uptake of 0.5 mM OMG in the presence of increasing concentrations of (●) cytochalasin B, (○) cytochalasin E, (■) genistein, and (□) myricetin. Uptake assays were done at room temperature with an average number of 4.7×10^7 cells per assay.

ylglucose (Figure 4E) but failed to affect the transport of nicotinamide (Figure 4D). As expected, control experiments revealed a lack of cytochalasin E effect on both nicotinamide and 3-*O*-methyl-D-glucose uptake (Figure 4, parts D and E).

Transport of Hexoses and Nicotinamide by CHO Cells Overexpressing GLUT1. These data indicate that, although nicotinamide may interact with GLUT1 as revealed by its effect on D-glucose-sensitive cytochalasin B binding, it is unable to affect the transport of hexoses by GLUT1. Similarly, the transport of nicotinamide was not affected by the hexoses that are GLUT1 substrates or by compounds that are GLUT1 inhibitors. The data suggest, therefore, that nicotinamide transport in human erythrocytes is through another transporter distinct from GLUT1. To further analyze this point, we studied the uptake of hexoses and nicotinamide in control and in transfected CHO cells overexpressing GLUT1. Uptake assays revealed that the transfected CHO cells transported 2-deoxy-D-glucose at a rate that was at least 5-fold higher than that of the control cells (Figure 5A). On the other hand, there was no difference in the uptake of nicotinamide in transfected cells as compared to control cells (Figure 5B), and nicotinamide failed to affect the uptake of 2-deoxy-D-glucose in both the control and transfected CHO cells (Figure 5C).

DISCUSSION

Using human erythrocytes, right-side-out and inside-out erythrocyte membrane vesicles, and GLUT1-transfected CHO cells, we show that nicotinamide is unable to inhibit the uptake of 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose, substrates of the facilitative hexose transporter GLUT1. We also show that the uptake of nicotinamide is unaffected by substrates and inhibitors of GLUT1 and that increased expression of GLUT1 is not associated with a corresponding increase in nicotinamide transport. Overall, our data suggest that nicotinamide is not a substrate of the GLUT1 transporter.

Our results are in opposition to those of Sofue et al. (*31*), who studied the uptake of glucose and nicotinamide in liposomes reconstituted with purified GLUT1. They reported that (a) liposomes exhibited saturable uptake of nicotinamide, (b) glucose inhibited the uptake of nicotinamide, and (c) nicotinamide inhibited the uptake of glucose and the binding of cytochalasin B. Our uptake data with normal human erythrocytes and right-side-out and inside-out erythrocyte membrane vesicles revealed that both the influx and the efflux of nicotinamide occurred in a time- and concentration-dependent manner. The influx of nicotinamide occurred with a K_M of 6.1 mM, while the K_M of the efflux was 6.2 mM,

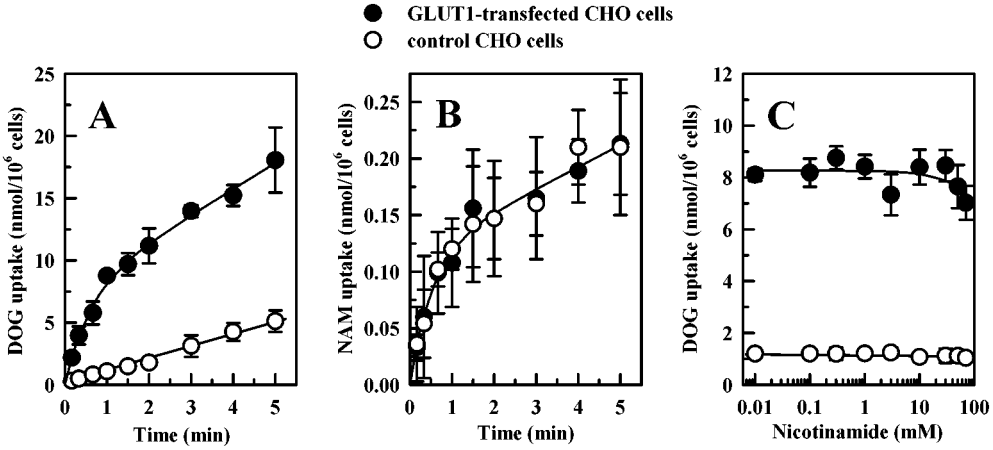

FIGURE 5: Uptake of 2-deoxy-D-glucose (DOG) and nicotinamide (NAM) into control and GLUT1-transfected CHO cells. (A) Time course of 0.5 mM DOG incorporation. (B) Time course of 1 mM nicotinamide incorporation. (C) Effect of nicotinamide on the uptake of DOG. Uptake assays were done at room temperature using an average number of 1×10^6 cells per assay.

which clearly indicates that in both cases the uptake of nicotinamide is mediated by a membrane transporter. Although these K_M values for transport are in the range reported by Sofue et al. in their reconstituted system (6.0−7.5 mM), our competition data do not support the concept that the nicotinamide transporter present in human erythrocytes and in the right-side-out and the inside-out erythrocyte vesicles is GLUT1. Thus, although the erythrocytes and the erythrocyte vesicles were capable of transporting 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose, two substrates that are transported by GLUT1, both hexoses failed to affect the uptake of nicotinamide at concentrations at which they clearly competed for the transport of each other. Moreover, nicotinamide did not affect the uptake of hexoses by both the erythrocytes and the erythrocyte vesicles. The highest concentration of nicotinamide used in these experiments (50 mM) exceeded those at which we observed saturation of uptake in the vesicles. On the other hand, inhibitors specific for GLUT1 completely blocked the uptake of hexoses by the erythrocytes and the erythrocyte vesicles, which makes it very implausible that the erythrocytes and the erythrocyte vesicles used in our studies would have had a specific defect in GLUT1 activity.

The effects we observed are not limited to erythrocytes or its membranes but were also seen in CHO cells overexpressing GLUT1; nicotinamide failed to block the uptake of hexoses in both control and GLUT1-transfected CHO cells. As expected, the increased expression of GLUT1 in the transfected CHO cells was accompanied by an enhanced capacity to take up 2-deoxy-D-glucose. On the other hand, this increased hexose transporting activity was not accompanied by a corresponding increase in nicotinamide uptake, revealing a lack of relationship between the level of GLUT1 expression and the transport of nicotinamide.

In reference to the results of Sofue et al., we could only confirm that nicotinamide was able to displace cytochalasin B from GLUT1 present in the erythrocyte membranes. Increasing concentrations of nicotinamide efficiently competed for the D-glucose-sensitive cytochalasin B binding sites on the erythrocyte membranes, therefore suggesting that nicotinamide effectively interacts with GLUT1. Although Sofue et al. did not analyze in detail the interaction between nicotinamide and GLUT1-bound cytochalasin B, we found that such displacement was noncompetitive, indicating an unlikelihood that nicotinamide would interact with a D-glucose recognition site on GLUT1. In additional support of this notion, cytochalasin B did not inhibit the uptake of nicotinamide by human erythrocytes. It is known that cytochalasin B binds at or near the endofacial sugar binding site in GLUT1, effectively blocking transport of substrates (*36*−*38*). As expected, in our experiments, cytochalasin B completely blocked the uptake of GLUT1 substrates.

It is not clear to us why our results diverge to such degree from those of Sofue et al., although a simple explanation may be that we used a very different experimental system. In the present study, we used whole cells (erythrocytes and CHO cells) or membrane preparations, while Sofue et al. used a reconstituted purified glucose transporter preparation. Although most of the reconstitution studies performed so far appear to support the notion that the reconstituted glucose transporter maintains most of its "native" properties, these studies have been restricted to the determination of glucose transport and cytochalasin B binding assays (*39*−*41*). Lacking additional information, we can only ask whether the addition of detergent (used in purifying the transporter) or artificial lipids (used in the reconstitution assays) may change the specificity of the glucose transporter in such a manner that could generate the different results reported by Sofue et al. and, hence, the conclusions.

Overall, our observations reaffirm the notion that under physiological conditions GLUT1 interacts with its substrates in a highly specific manner which includes the recognition in the substrate of a pyranose or furanose ring with the proper chirality (*1*). From our results, either the presence of an aromatic pyridine ring or the meta position of the carboxamide side chain in nicotinamide preclude its interaction with the substrate recognition sites of GLUT1. Therefore, under our experimental conditions it is unlikely that GLUT1 transports nicotinamide because it would have to bind to a site different from that for sugars and would have to traverse a pathway different from that for sugars. In conclusion, our data suggests that nicotinamide crosses the cell membrane through a transporter altogether different from GLUT1.

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