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Suppressed intrinsic catalytic activity of GLUT1 glucose transporters in insulin-sensitive 3T3-L1 adipocytes

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ABSTRACT **Previous studies indicated that the erythroid-type (GLUT1) glucose transporter isoform contributes to basal but not insulin-stimulated hexose transport in mouse 3T3-L1 adipocytes. In the present studies it was found that basal hexose uptake in 3T3-L1 adipocytes was about 50% lower than that in 3T3-L1 or CHO-K1 fibroblasts. Intrinsic catalytic activities of GLUT1 transporters in CHO-K1 and 3T3-L1 cells were compared by normalizing these hexose transport rates to GLUT1 content on the cell surface, as measured by two independent methods. Cell surface GLUT1 levels in 3T3-L1 fibroblasts and adipocytes were about 10- and 25-fold higher, respectively, than in CHO-K1 fibroblasts, as assessed with an anti-GLUT1 exofacial domain antiserum, delta. The large excess of cell surface GLUT1 transporters in 3T3-L1 adipocytes relative to CHO-K1 fibroblasts was confirmed by GLUT1 protein immunoblot analysis and by photoaffinity labeling (with 3-[^{125}I]iodo-4-azidophenethylamido-7-*O*-succinyldeacetylforskolin) of glucose transporters in isolated plasma membranes. Thus, GLUT1 intrinsic activity is markedly reduced in 3T3-L1 fibroblasts compared with the CHO-K1 fibroblasts, and further reduction occurs upon differentiation to adipocytes. Intrinsic catalytic activities specifically associated with heterologously expressed human GLUT1 protein in transfected CHO-K1 versus 3T3-L1 cells were determined by subtracting appropriate control cell values for hexose transport and delta-antibody binding from those determined in the transfected cells expressing high levels of human GLUT1. The results confirmed a >90% inhibition of the intrinsic catalytic activity of human GLUT1 transporters on the surface of mouse 3T3-L1 adipocytes relative to CHO-K1 fibroblasts. We conclude that a mechanism that markedly suppresses basal hexose transport catalyzed by GLUT1 is a major contributor to the dramatic insulin sensitivity of glucose uptake in 3T3-L1 adipocytes.**

Stimulation of cellular glucose transport rates by insulin is critically important to the regulation of mammalian glucose metabolism. Hormonally responsive adipocytes are excellent cells in which to study this effect, due to the 15- to 30-fold stimulations of glucose transport rates elicited by insulin in those cells (for review, see ref. 1). Two such well-studied model systems, isolated rat adipocytes and cultured mouse 3T3-L1 adipocytes, express a skeletal muscle/adipocyte-type (GLUT4) glucose transporter isoform that appears to play a major role in the responses of those cells to insulin (2–10). However, recent reports indicate that 3T3-L1 adipocytes also express relatively high levels of the erythroid-type (GLUT1) glucose transporter and that a significant proportion of that protein resides at the cell surface under normal cell culture conditions (7, 8, 10–12). In spite of the apparent abundance of these cell surface transporters, 3T3-L1 adipocytes exhibit relatively low basal sugar-transport rates compared with human erythrocytes (1) or Chinese hamster ovary (CHO) fibroblasts (13, 14). These observations suggest the possibility that intracellular environment modulates the intrinsic catalytic activity of the GLUT1 glucose transporter protein in 3T3-L1 adipocytes.

To test the hypothesis that GLUT1 transporters are inhibited in 3T3-L1 adipocytes, we compared the relative levels of GLUT1 protein on the surfaces of intact CHO fibroblasts, undifferentiated 3T3-L1 fibroblasts, and differentiated 3T3-L1 adipocytes with their relative rates of hexose transport. Although the primary structures of GLUT1 proteins in other rodent cells (mouse, rat) and in rabbit cells are known to be >98% identical (6, 13, 15), the primary structure of the hamster GLUT1 has not been determined. Therefore, transfected CHO fibroblast and 3T3-L1 adipocyte cell lines expressing high levels of human GLUT1 were utilized to determine whether heterologously expressed human GLUT1 protein was inhibited in the adipocytes relative to the fibroblasts. We report a >90% suppression of GLUT1 transporter catalytic activity in the differentiated 3T3-L1 adipocytes relative to the cultured fibroblasts.

METHODS

Cell Cultures. CHO-K1 and mouse 3T3-L1 fibroblasts were grown in culture as described (8, 14). Two days after the 3T3-L1 fibroblasts achieved confluence, differentiation to adipocytes was induced (7, 8). After induction of differentiation, the cells were maintained for 10–14 days prior to measurement of sugar uptake or harvesting of cell membranes.

Assay of Hexose Transport. 2-Deoxyglucose uptake and 3-*O*-methylglucose influx in CHO-K1 fibroblasts and 3T3-L1 fibroblasts and adipocytes were assayed as described (8, 14, 16). The numbers of cultured cells were determined for each experiment, and the data were normalized per 10^6 cells.

Immunoblot Analysis of Plasma Membrane Glucose Transporter. Total membrane and plasma membrane fractions were prepared from CHO-K1 fibroblasts and 3T3-L1 adipocytes, and the membrane proteins were solubilized in sample buffer, resolved by SDS/10% PAGE (17), and transferred to nitrocellulose, as described (8, 14). Immunoblot analyses of GLUT1 protein levels were performed using rabbit anti-GLUT1 C-terminal peptide antiserum (R495, East Acres Biologicals, Southbridge, MA; 1:1000 dilution) and ^{125}I-labeled protein A (1:500 dilution). ^{125}I was detected by autoradiography and quantitated using an LKB Ultroscan XL enhanced laser densitometer.

Photoaffinity Labeling of Glucose Transporter Protein. CHO-K1 fibroblast and 3T3-L1 adipocyte membranes were labeled with 3-[^{125}I]iodo-4-azidophenethylamido-7-*O*-succinyldeacetylforskolin ([^{125}I]IAPS-forskolin; refs. 18 and 19). Membrane proteins (200 μg) were incubated with [^{125}I]IAPS-forskolin in the presence or absence of 200 μM

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Abbreviation: [^{125}I]IAPS-forskolin, 3-[^{125}I]iodo-4-azidophenethylamido-7-*O*-succinyldeacetylforskolin.

cytochalasin B for 30 min at room temperature, in the dark. The protein/ligand mixtures were then diluted to a final volume of 5 ml and photolyzed for 5 sec under a high-intensity UV lamp. 2-Mercaptoethanol was then added to the reaction mixture (1% final concentration, vol/vol), the membranes were pelleted by centrifugation at 200,000 × g for 1 hr, and the membrane proteins were solubilized and resolved by SDS/PAGE as described above. Polyacrylamide gels were then dried under vacuum. ^{125}I was detected by autoradiography and quantitated using an LKB Ultroscan XL enhanced laser densitometer.

Binding of Anti-Glucose Transporter Antibody to Intact Cells. Control and transfected CHO and 3T3-L1 cells grown in culture were assayed for delta-antibody binding as described (14, 29). Nonspecific binding (preimmune serum) was subtracted from total binding for each cell line. Each assay point was determined in quadruplicate.

RESULTS

To test the hypothesis that the catalytic activity of the GLUT1 glucose transporter is suppressed in mouse 3T3-L1 adipocytes, the relative rates of sugar transport and the levels of cell surface GLUT1 protein in these cells were compared with those in CHO-K1 and mouse 3T3-L1 fibroblasts (Table 1). Sugar transport rates in intact cells were determined by assaying 2-deoxyglucose accumulation at 37°C and by assaying 3-*O*-methylglucose influx at room temperature. Cell surface GLUT1 protein levels were also determined in intact cells by measuring binding of a GLUT1-specific antibody, delta, that recognizes one or more extracellular epitopes on the GLUT1 glucose transporter (14). This antibody binds quantitatively to intact erythrocytes, to intact CHO-K1 fibroblasts expressing human GLUT1, and to rightside-out erythrocyte ghosts, but not to inside-out erythrocyte ghosts (14). Additionally, binding of delta antibody to intact 3T3-L1 fibroblasts and adipocytes is directly proportional (1:1) to plasma membrane and total cellular membrane GLUT1 protein levels over a 7-fold range (29). Hexose transport rates were similar in the two fibroblast cell lines and 2-fold higher than in 3T3-L1 adipocytes. In contrast, the levels of cell surface GLUT1 protein bound by delta antibody were 2.6-fold higher in the 3T3-L1 adipocytes than in the 3T3-L1 fibroblasts and ≈30-fold higher in the adipocytes than in the CHO-K1 fibroblasts (Table 1).

When the cellular transport rates described above are normalized to delta-antibody binding, it is apparent that cell surface GLUT1 proteins in 3T3-L1 and CHO-K1 fibroblasts transport sugar at 6 times and >60 times the rate of 3T3-L1 adipocytes, respectively. These hexose transport/delta-antibody binding ratios suggest that cell surface mouse GLUT1 proteins in 3T3-L1 cells are >95% inhibited relative to hamster GLUT1 proteins present at the surface of CHO-K1 cells. Furthermore, 3T3-L1 adipocyte GLUT1 proteins appear to be >80% inhibited relative to 3T3-L1 fibroblast GLUT1 proteins. Note that the influx rates measured using the nonmetabolizable glucose analog 3-*O*-methylglucose indicate that potential differences in the rates of sugar metabolism cannot account for the differences in GLUT1 catalytic activities observed between the CHO-K1 fibroblasts and the 3T3-L1 adipocytes.

Immunoreactive GLUT1 Protein in Plasma Membrane Fractions from CHO-K1 Fibroblasts and 3T3-L1 Adipocytes. A second analytical approach was used to determine whether the relative cell surface GLUT1 levels measured by the delta-antibody binding assay could be confirmed. Plasma membrane proteins were harvested from CHO-K1 fibroblasts and mouse 3T3-L1 adipocytes as described under *Methods*. Cellular homogenates and membrane fractions were assayed for activity of the plasma membrane marker enzyme 5′-nucleotidase (7). Plasma membrane fractions from CHO-K1 fibroblasts were ≈7-fold enriched in 5′-nucleotidase activity, and plasma membrane fractions from 3T3-L1 adipocytes were 7- to 9-fold enriched in that enzyme marker activity, relative to total cellular homogenates. Approximately 25% of the total homogenate 5′-nucleotidase activity was recovered in the plasma membrane fractions of each cell line.

GLUT1 protein levels were compared by immunoblot analysis of these two plasma membrane fractions with an antiserum prepared against the C-terminal peptide of GLUT1. Immunoblot data in Fig. 1 are representative of experiments comparing a total of six and four different preparations of membrane proteins from 3T3-L1 adipocytes and CHO-K1 fibroblasts, respectively. The amounts of membrane proteins loaded onto each lane of the polyacrylamide gel were calculated to reflect the relative levels of plasma membrane GLUT1 protein harvested on a per-cell basis. Culture plates (150 mm) of 3T3-L1 adipocytes contained 4-fold more plasma membrane protein but 50% fewer cells than the same-size culture plates of the hamster fibroblasts. Thus, the amounts of membrane protein analyzed reflect that 8-fold correction factor. In Fig. 1*A*, lanes 1–4 contained 100 μg each of 3T3-L1 adipocyte membrane protein and lanes 5–7 contained 12.5 μg each of hamster fibroblast membrane protein. Autoradiographs were scanned by laser densitometry to determine the relative amounts of GLUT1 protein in each lane (Fig. 1*B*). This method of quantitation indicated that the average level of plasma membrane fraction GLUT1 protein per cell was 7-fold higher in 3T3-L1 adipocytes (3.0 ± 0.18, $n = 6$ preparations) than in CHO-K1 fibroblasts (0.43 ± 0.12, $n = 4$ preparations). Thus, these data, combined with the 2-fold higher transport rates measured in the CHO-K1 cells (Table 1), yield an estimated intrinsic catalytic activity for these cells that is ≈14-fold higher than that of 3T3-L1 adipocytes.

Total Photoaffinity-Labeled Glucose Transporters in CHO-K1 Fibroblasts Versus 3T3-L1 Adipocytes. It is possible that other glucose transporter isoforms are present in CHO-K1 fibroblasts and that these proteins account for the relatively high hexose transport rates in Table 1. Therefore,

Table 1. Hexose transport rates versus cell surface GLUT1 protein levels

Cells	dGlc uptake, pmol/min per 10^6 cells	3-*O*-MeGlc influx, pmol/min per 10^6 cells	δ-Ab binding, cpm × 10^{-3} per 10^6 cells	dGlc uptake/δ-Ab binding	3-*O*-MeGlc influx/δ-Ab binding
CHO-K1 fibroblasts	125 ± 11	12 ± 2	0.421 ± 0.078	297	29
3T3-L1 fibroblasts	112 ± 17	ND	4.94 ± 0.57	22.7	ND
3T3-L1 adipocytes	52 ± 5	5.6 ± 1.4	13.0 ± 1.3	4.0	0.43

2-Deoxyglucose (dGlc, 100 μM) uptake data are averages of 19, 21, and 16 experiments for CHO-K1 fibroblasts, 3T3-L1 fibroblasts, and 3T3-L1 adipocytes, respectively. 3-*O*-Methylglucose (3-*O*-MeGlc, 50 μM) influx data are averages of 5 and 4 experiments for CHO-K1 fibroblasts and 3T3-L1 adipocytes, respectively. delta-Antibody (δ-Ab) binding data are averages of 7, 6, and 6 experiments for CHO-K1 fibroblasts, 3T3-L1 fibroblasts, and 3T3-L1 adipocytes, respectively. ND, not done.

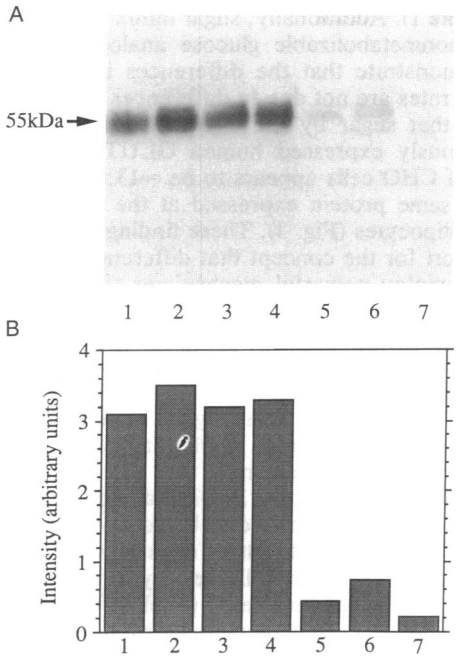

FIG. 1. Plasma membrane GLUT1 immunoreactive protein in CHO-K1 fibroblasts and mouse 3T3-L1 adipocytes. CHO-K1 fibroblasts and mouse 3T3-L1 adipocytes were grown on 150-mm culture dishes and plasma membrane fractions were harvested from these cells. (*A*) Adipocyte (lanes 1–4) and CHO-K1 fibroblast (lanes 5–7) plasma membrane proteins from $\approx 10^7$ cells (100 μg and 12.5 μg, respectively) were solubilized, resolved by SDS/PAGE, and transferred to nitrocellulose filters. Protein immunoblot analysis was performed using rabbit anti-GLUT1 C-terminal peptide antiserum and ^{125}I-protein A. GLUT1 proteins were visualized by autoradiography. These data represent three and four different plasma membrane preparations from CHO-K1 fibroblasts and 3T3-L1 adipocytes, respectively. (*B*) Relative intensities of the immunoreactive GLUT1 band in lanes 1–7 were determined by scanning densitometry.

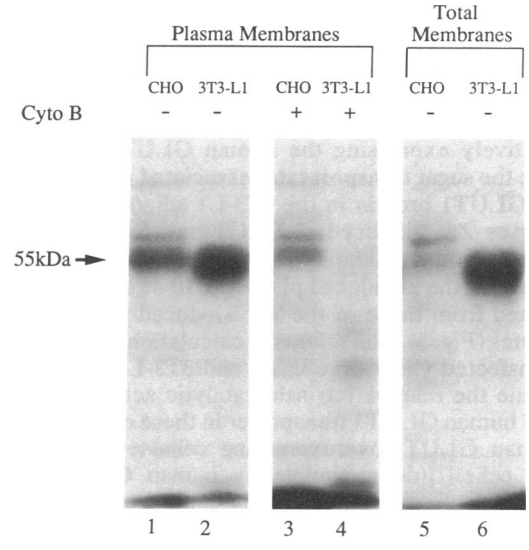

FIG. 2. Relative levels of [^{125}I]IAPS-forskolin-labeled glucose transporter protein in CHO-K1 fibroblasts and mouse 3T3-L1 adipocytes. CHO-K1 fibroblasts and mouse 3T3-L1 adipocytes were grown on 150-mm culture dishes and total cellular membranes and plasma membrane fractions were harvested. Two hundred micrograms of membrane protein harvested from each cell type was photoaffinity-labeled with [^{125}I]IAPS-forskolin in the presence (lanes 3 and 4) or absence (lanes 1, 2, 5, and 6) of 200 μM cytochalasin B (Cyto B). The membranes were pelleted by centrifugation, and membrane proteins were solubilized and resolved by SDS/PAGE. ^{125}I was detected by autoradiography. Results from a representative experiment are presented. Total glucose transporter proteins from CHO-K1 plasma membranes (lanes 1 and 3) and 3T3-L1 adipocyte plasma membranes (lanes 2 and 4) and from total membranes of CHO-K1 fibroblasts and 3T3-L1 adipocytes (lanes 5 and 6, respectively) were labeled with [^{125}I]IAPS-forskolin.

an independent method was utilized to compare the total plasma membrane glucose transporter protein levels in these fibroblasts and in the mouse 3T3-L1 adipocytes. Plasma membrane proteins harvested from each cell type were covalently labeled with the photoaffinity label [^{125}I]IAPS-forskolin and separated by SDS/PAGE (Fig. 2). This transport inhibitor has been shown to selectively label glucose transporters from various cell types including human red cells (18), isolated rat adipocytes (5, 19), and mouse 3T3-L1 cells (7, 8, 29). Approximately 2- to 3-fold more glucose transporter protein was labeled by [^{125}I]IAPS-forskolin in the adipocyte plasma membranes than in the hamster fibroblast plasma membranes when equal amounts of protein were analyzed (Fig. 2, lanes 1 and 2). [^{125}I]IAPS-forskolin labeling was inhibited by the general glucose transport inhibitor cytochalasin B (lanes 3 and 4). Thus, the 8-fold correction factor described above was used to normalize the data to a per-cell basis, and 16–24 times more transporter protein was found in the adipocyte plasma membrane fraction than in the corresponding fibroblast plasma membranes. Similarly, [^{125}I]IAPS-forskolin-labeled glucose transporters in total cellular membranes from each cell type showed no indication that CHO-K1 fibroblasts express significant levels of another transporter isotype (Fig. 2, lanes 5 and 6). The data in Table 1 and in Figs. 1 and 2 indicate that the 3T3-L1 adipocytes maintain lower rates of sugar transport but higher levels of cell surface glucose transporter protein than the CHO-K1 fibroblasts.

Hexose Transport and delta-Antibody Binding in Transfected Cells Expressing Human GLUT1. Although rodent and rabbit GLUT1 proteins appear to be >98% identical (6, 13, 15), the primary structure of the CHO cell GLUT1 protein has not been determined, and it is possible that significant structural differences between the GLUT1 proteins expressed in these cultured cells account for the different catalytic activities. To test directly the hypothesis that the catalytic activity of GLUT1 transporters is inhibited when the protein is expressed in mouse 3T3-L1 adipocytes, CHO-K1 fibroblasts and mouse 3T3-L1 adipocytes transfected with cDNAs encoding the human GLUT1 protein were used. The relative contributions of the expressed human GLUT1 protein to the levels of cell surface transporters and sugar transport rates in the two cell types were determined. Note that the expression vector used in these studies, pLENGT, contains the metal-inducible human metallothionein II promoter. The transfected CHO-K1 fibroblast cell line used in these studies, CHO-GT3 (14), constitutively expresses human GLUT1 protein at 20- to 40-fold higher levels than those of hamster host GLUT1 protein; therefore, there was no need to induce additional protein expression with heavy metal. The mouse 3T3-L1 adipocyte cell line, pLENGT 10-45 (8), constitutively expresses high absolute levels of human GLUT1 protein. However, this yields only a 2-fold increase in total cellular GLUT1, due to the high levels of endogenous mouse GLUT1 protein present in these cells. Therefore, treatment of the pLENGT 10-45 adipocytes for 18 hr with 125 μM Zn^{2+} was employed to exert a 3- to 4-fold induction of human GLUT1 protein expression (8). Treatment of untransfected 3T3-L1 adipocytes with Zn^{2+} causes small, 0–50% increases in mouse GLUT1 protein levels (8), and no increase in binding of delta antibody to the untransfected adipocytes was observed under these experimental conditions (data not shown).

delta-Antibody binding and sugar transport rates associated with the heterologously expressed human transporter protein were determined by subtracting the antibody binding values and the sugar transport rates measured for the parental CHO-K1 fibroblasts from those of CHO-GT3 fibroblasts constitutively expressing the human GLUT1 (Fig. 3). To measure the sugar transport rates associated with cell surface human GLUT1 protein in the 3T3-L1 adipocytes, constitutive versus Zn^{2+}-induced pLENGT-transfected cells were examined. Antibody binding values and sugar transport rates measured for the uninduced pLENGT 10-45 adipocytes were subtracted from those of the Zn^{2+}-induced pLENGT 10-45 adipocytes (Fig. 3). The following calculations were made for the transfected CHO fibroblasts and 3T3-L1 adipocytes to determine the relative intrinsic catalytic activity of the expressed human GLUT1 transporter in these cells: [(transport by human GLUT1-overexpressing cells) − (transport by control cells)]/[(delta binding by human GLUT1-overexpressing cells) − (delta binding by control cells)] = Human GLUT1 transport/cell surface human GLUT1.

The ratios of 2-deoxyglucose uptake to delta-antibody binding determined by this method are 0.20 for the CHO fibroblasts versus 0.016 for the mouse 3T3-L1 adipocytes, consistent with a 92% inhibition of human GLUT1 catalytic activity in those adipocytes.

DISCUSSION

Data reported in this paper indicate that the intrinsic catalytic activity of cell surface GLUT1 glucose transporters in 3T3-L1 adipocytes is suppressed by at least 80–90% compared with that of GLUT1 proteins expressed in CHO-K1 or 3T3-L1 fibroblasts. Two independent methods using three independent labeling reagents all indicate that the cultured mouse adipocytes express significantly higher levels of cell surface glucose transporter protein than do CHO-K1 fibroblasts. However, the 3T3-L1 adipocytes exhibit lower rates of 2-deoxyglucose influx than the fibroblasts on a per-cell

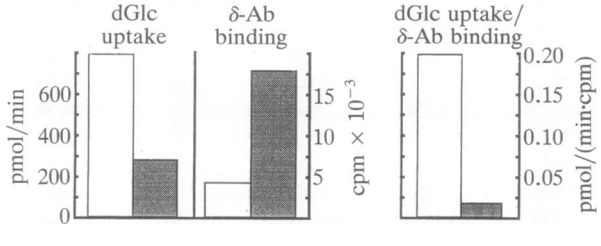

FIG. 3. Relative effects of human GLUT1 protein expression on hexose transport and delta-antibody binding in transfected CHO-K1 fibroblasts and mouse 3T3-L1 adipocytes. Parental CHO-K1 fibroblasts, transfected CHO-K1 fibroblasts overexpressing human GLUT1 protein (CHO-GT3 cell line), and transfected mouse 3T3-L1 adipocytes overexpressing human GLUT1 protein (pLENGT 10-45 cell line) were grown in multiwell culture dishes. CHO fibroblast cell lines were assayed for 100 μM 2-deoxyglucose (dGlc) uptake, and delta-antibody (δ-Ab) binding by intact cells was assayed using a 1:500 dilution of rabbit anti-human erythrocyte GLUT1 antiserum δ and a 1:500 dilution of ^{125}I-protein A. Note that pLENGT adipocytes were assayed using identical procedures, except that prior to washing and serum starvation, these cultured adipocytes were incubated for 18 hr with or without 125 μM zinc sulfate, which induces optimum expression of human GLUT1 protein (8). Hexose uptake rates and delta-antibody binding due to the expressed human protein were determined by (*i*) subtracting the parental CHO-K1 cell uptake and delta-antibody binding values from those of the CHO-GT3 cells (open bars) and (*ii*) subtracting the untreated (noninduced) pLENGT adipocyte uptake and delta-antibody binding values from those of the 125 μM Zn^{2+}-treated (induced) pLENGT adipocytes (shaded bars). Values shown are for 10^6 cells. These difference data were replotted as ratios of hexose uptake to delta-antibody binding for the hamster fibroblasts and the mouse adipocytes.

basis (Table 1). Additionally, sugar influx studies performed using a nonmetabolizable glucose analog, 3-*O*-methylglucose, demonstrate that the differences in 2-deoxyglucose transport rates are not due to differences in cellular metabolism of that sugar by the two cell types. Furthermore, heterologously expressed human GLUT1 protein on the surface of CHO cells appears to be ≈13 times more active than the same protein expressed at the surface of mouse 3T3-L1 adipocytes (Fig. 3). These findings provide compelling support for the concept that differentiated 3T3-L1 adipocytes employ powerful mechanisms to restrain GLUT1 transport activity.

Calculations made from measurements of delta-antibody binding to intact cells (Table 1) yielded quite similar estimations of 3T3-L1 adipocyte GLUT1 suppression when compared with immunoblot data (Fig. 1) obtained with isolated plasma membrane fractions (99% versus 93% suppression, respectively). It is noteworthy that the increment in cell surface GLUT1 levels in 3T3-L1 adipocytes relative to CHO-K1 cells was estimated to be somewhat greater when delta-antibody binding to intact cells was used rather than membrane fractionation followed by protein immunoblot analysis. This might be due to possible differences in the contamination of plasma membrane fractions prepared from CHO-K1 cells versus 3T3-L1 adipocytes. However, both the yield and the enrichment of 5′-nucleotidase in plasma membranes from these two cell types were similar. Another potential error could result from differences between the cell lines in the nonspecific binding of immunoglobulins in the delta-antibody preparations. However, by comparing CHO-K1 and 3T3-L1 cell lines hyperexpressing human GLUT1 protein with appropriate control cells, this problem is theoretically eliminated. In any case, the results obtained with both approaches lead to the conclusion that there is >90% suppression of GLUT1 activity in 3T3-L1 cells versus CHO-K1 cells.

It is possible that other glucose transporter isoforms present in CHO or 3T3-L1 cells confound this interpretation. [^{125}I]IAPS-forskolin photoaffinity labeling of total glucose transporters was used to address this issue (Fig. 2). This reagent, like cytochalasin B, binds to a glucose binding site that does not appear to be specific for any one isoform. The labeling of glucose transporters in CHO-K1 plasma membranes with this reagent was about 20-fold lower on a per-cell basis than that in 3T3-L1 adipocyte plasma membranes (Fig. 2), even though transport activity was twice as high in the former cell type. It is unlikely that there is in CHO-K1 cells a glucose transporter type that contributes to the high cellular glucose transport activity but escapes photolabeling with this reagent. We found in preliminary experiments that 100% of the cytochalasin B-sensitive 2-deoxyglucose uptake in CHO-K1 cells was also inhibited by forskolin (data not shown). Thus, virtually all of the transporter proteins present in these cells bind this inhibitor and are expected targets for photolabeling by [^{125}I]IAPS-forskolin.

A significant fraction of the 20-fold excess affinity-labeling of 3T3-L1 adipocyte plasma membrane glucose transporters over that of CHO-K1 membranes can be ascribed to the presence of GLUT4 in the former cell type (3, 6–8, 10). This glucose transporter isoform has been reported to comprise 25–50% of the total GLUT1 plus GLUT4 transporters in differentiated 3T3-L1 adipocytes (10, 20). When this predicted GLUT4 component was subtracted from the total photolabel incorporated into 3T3-L1 adipocyte membranes, the remaining excess photolabel over that measured in CHO-K1 membranes was calculated as 10- to 15-fold. This compares with a 7-fold excess of GLUT1 immunoreactivity measured in 3T3-L1 adipocyte versus CHO-K1 fibroblast plasma membranes (Fig. 1). Also, note that the estimates of GLUT1 suppression in 3T3-L1 adipocytes versus CHO-K1

cells based on the relative levels of forskolin-labeled transporter protein (95–98% suppression) are virtually identical to those based on delta-antibody binding data.

The hypothesis that GLUT1 glucose transporter activity is suppressed in 3T3-L1 adipocytes provides a plausible explanation for the apparent incongruity between the higher levels of GLUT1 relative to GLUT4 in these cells, which are highly sensitive to insulin, and the idea that GLUT4 proteins are primarily responsible for insulin-stimulated glucose transport in these cells (8). Estimates of the GLUT1 protein present in the cell surface membranes of control 3T3-L1 adipocytes range from ≈20% (11) to 50% (7, 8, 16, 21) of the total cellular GLUT1. Thus, even in the basal state there appears to be nearly as much cell surface GLUT1 as there is total cellular GLUT4. Recruitment of GLUT4 to the plasma membrane by insulin would not be sufficient to provide the observed 15-fold increase in glucose transport rates, if all transporters were equally active. In the presence of >90% suppression of GLUT1 activity, however, this effect of insulin on GLUT4 could account for the observed glucose transport stimulation. Our data, which suggest a >90% suppression of GLUT1 activity in 3T3-L1 adipocytes, are consistent with this concept.

The hypothesis that glucose transporter intrinsic catalytic activity can be markedly altered in various cell types is strongly supported by several studies. Modulation of glucose transporter intrinsic activity by the counterregulatory action of catecholamines on insulin-stimulated sugar transport in rat adipocytes (22, 23) and by ATP in human erythrocytes (24–26) is established. Exposures of 3T3-L1 adipocytes to a number of agents appear to reveal latent glucose transport activity. Exposure of these adipocytes to cholera toxin or dibutryl cAMP (7), protein synthesis inhibitors (16), or low micromolar concentrations of Cd^{2+} (29) dramatically increase hexose transport rates without recruiting additional GLUT1 or GLUT4 proteins to the cell surface. The present data indicate that a possible mechanism for such glucose transport activation is the reversal of the GLUT1 suppression that appears to occur in 3T3-L1 adipocytes.

The data presented here suggest that there are at least two mechanisms by which 3T3-L1 adipocytes may regulate glucose transport. The cultured adipocytes appear to maintain low rates of basal hexose influx both by sequestering transporters in intracellular pools and by inhibiting cell surface glucose transport proteins and are thus poised for a dramatic stimulation of sugar transport by insulin. A number of cellular treatments or stresses can relieve the transporter inhibition without a concomitant recruitment of transporter proteins to the cell surface (see preceding paragraph). Therefore, modulation of cell surface glucose transporter catalytic activity may represent a general type of sugar transport regulation, in addition to the well-described recruitment of cellular transporters (1, 27) and the induction of *de novo* transporter protein synthesis (28).

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