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Detection of the Rat Adipose Cell Glucose Transporter with Antibody Against the Human Red Cell Glucose Transporter

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[^{125}I]-protein A and an affinity purified rabbit antibody against the purified human erythrocyte glucose transporter have been used to label rat adipose cell plasma and low-density microsomal membrane proteins after transfer from SDS-polyacrylamide gels to nitrocellulose paper. A major labeled band is observed in both membrane preparations with an apparent molecular weight (approximately 45,000) similar to that of the human erythrocyte glucose transporter. When membranes are prepared from cells incubated in the absence of insulin, the intensity of this band is roughly 2-fold greater in the microsomal membranes than in the plasma membranes. Incubation of cells with a maximally stimulating concentration of insulin, however, increases the intensity of this band in the plasma membranes and concomitantly decreases that in the microsomal membranes. In both cases, the intensity of this band roughly parallels the concentration of glucose transport systems determined by specific D-glucose-inhibitable [^{3}H]cytochalasin B binding. These results indicate that the rat adipose cell glucose transporter is immunologically similar to the human erythrocyte glucose transporter.

Recent studies have demonstrated that insulin stimulates glucose transport activity in rat adipose (1-4) and muscle (5) cells primarily through the translocation of glucose transporters from an intracellular membrane pool to the plasma membrane. However ,the adipose and muscle cell glucose transporters have not yet been identified.

The glucose transporter from human erythrocytes has been purified and shown to be a glycoprotein with an apparent molecular weight of about 55,000 (6-8). Furthermore rabbit antisera against the purified human erythrocyte transporter have been prepared in two laboratories (9,10). The antisera react with the

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purified transporter and a broad 55,000 MW band in freshly prepared erythrocyte ghosts. In one study (10) the antibody was shown to cross-react with a similar molecular weight protein in plasma membranes from HeLa cells and to cross-react with mouse L-1210 cells. The latter results indicate a potential usefulness of these antisera as probes for the glucose transporter in other tissues and species.

In this report, the technique of electrophoretic transfer of proteins from SDS-polyacrylamide gels to nitocellulose paper (11) followed by antibody and [^{125}I]-protein A labeling (12) has been used to identify a cross-reacting protein of approximately 45,000 MW in rat adipose cell plasma and microsomal membrane fractions. In addition, changes in the amount of cross-reacting protein in response to insulin are shown to parallel changes in the concentration of glucose transporters measured by cytochalasin B binding.

EXPERIMENTAL PROCEDURES

Preparations: Isolated rat epididymal adipose cells were prepared and incubated in the absence or presence of 7.0 nM (1000 μU/ml) insulin as previously described (1,3). Plasma and low-density microsomal membrane fractions were prepared by differential ultracentrifugation and the number of D-glucose-inhibitable cytochalasin B binding sites was measured as described (1,3). Human erythrocyte glucose transporter (13,6) and erythrocyte ghosts (14) were prepared as described. Antibody against the human erythrocyte glucose transporter was prepared by affinity chromatography using SDS-denatured purified transporter as described (10).

Visualization of cross-reacting membrane proteins: SDS-polyacrylamide slab gels (7.5% polyacrylamide) were run using the procedure of Laemmli (15). The proteins were transferred electrophoretically to nitrocellulose paper (Schleicher and Schuell) as described by Towbin et al. (11) in 25 mM Tris, 192 mM glycine and 20% methanol for 6 h at 200 mA.

Cross-reacting protein was visualized after transfer using the antibody against the human erythrocyte glucose transporter, [^{125}I]-protein A and a procedure similar to that described by Renart et al. (12). All washes and incubations were done at 4°. The nitrocellulose sheet was washed 3 times for 30 min with 150 ml of a solution of 10 mM Tris, 1 mM EDTA, 150 mM NaCl and 0.1% Triton X-100, pH 7.4 (rinse buffer). It was then washed with 100 ml of rinse buffer containing 30 mg bovine serum albumin/ml for 2 h. The sheet was sealed in a Seal-A-Meal bag (Dazey) with 4 ml rinse buffer containing 12 mg bovine serum albumin and 20 μg antibody and incubated for 14 h. It was then washed 3 times for 30 min with 150 ml of rinse buffer, sealed in a bag with 4 ml of rinse buffer containing about 20 μCi of [^{125}I]-protein A and incubated for 6 h. After this incubation it was washed 3 times for 30 min with 150 ml of 10 mM Tris, 1 mM EDTA, 1 M NaCl and 0.1% Triton X-100 and allowed to dry. Autoradiography was done at -20° using Kodak XAR-5 film and Dupont Cronex Lightning-Plus intensifying screens. For quantitation, the autoradiograms were cut into strips and scanned using a Millipore PhoroScope densitometer.

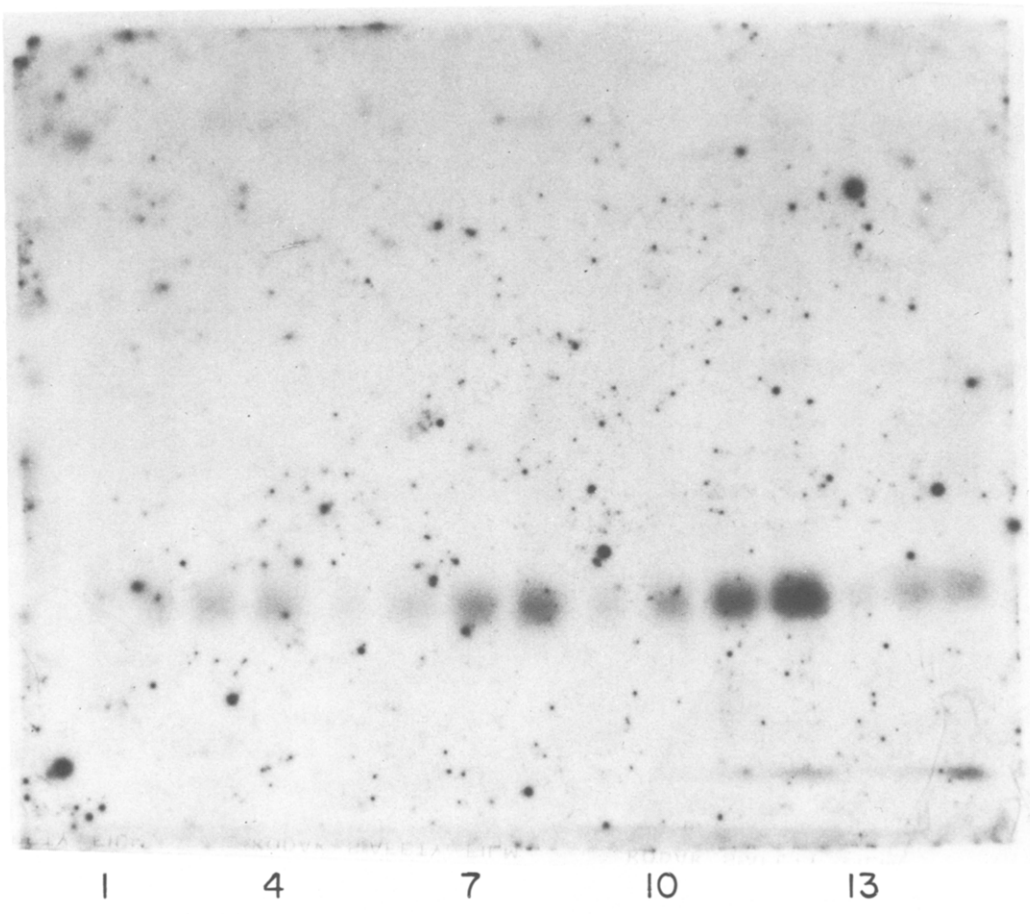

Fig. 1. Autoradiogram of Transferred Proteins from Rat Adipose Cell Membranes. Labeling was performed using the antibody to the purified human erythrocyte transporter and [^{125}I]-protein A. Plasma membranes: lanes 1-4, basal; (10,20, 30,40 µg); lanes 5-8, insulin-treated (10,20,30,40 µg). Low density microsomal membranes: lanes 9-12, basal (10,20,40,60 µg); lanes 13-15, insulin-treated (20,40,60 µg).

RESULTS AND DISCUSSION

Labeling of Adipose Cell Membrane Fractions. Fig. 1 shows the labeling patterns observed with gel transfers of plasma and low-density microsomal membranes prepared from basal and maximally insulin-stimulated isolated rat adipose cells. A major labeled band is observed in both membrane preparations regardless of the absence or presence of insulin. Similar gels including molecular weight standards indicate an apparent molecular weight for this band of about 45,000. Other very faint bands are sometimes seen, although they are not visible in the gel shown in Fig. 1. They correspond to major proteins seen by

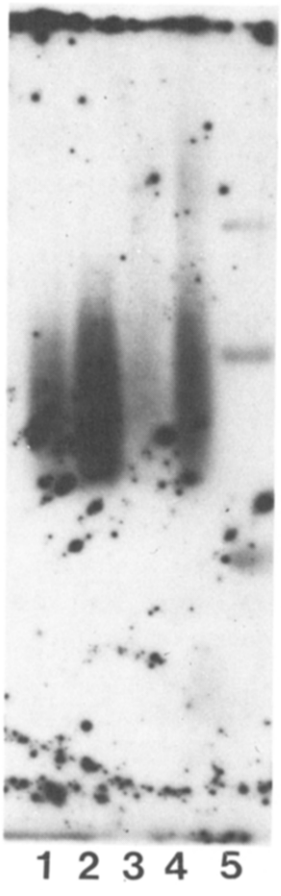

Fig. 2. Autoradiograms of Electrophoretic Protein Transfers of Human Erythrocyte Glucose Transporter and Ghost Membranes. Labeling was performed as in Fig. 1. Lanes 1-2, ghosts prepared from fresh erythrocytes, 0.2 and 0.4 µg. Lanes 3-4, purified transporter, 0.02 and 0.04 µg protein. Lane 5 marker polypeptides showing nonspecific staining, 11 µg: lactic dehydrogenase (35 K), catalase (58 K), and phosphorylase (92 K).

Coomassie blue staining and probably represent nonspecific labeling due to the relatively high protein loads and long exposure times necessary to visualize the major band.

Autoradiograms of transfers from SDS-polyacrylamide gels of the purified human erythrocyte glucose transporter and ghosts prepared from fresh erythrocytes are shown in Fig. 2 for comparison. In each case, a broad band centered at about 55,000 MW is labeled by the antibody. The broadness is due to variable glycosylation of the protein and the polypeptide alone has an apparent molecular weight of 46,000 (16). The high sensitivity of the protein transfer method is shown by the fact that only 0.02 µg of purified transporter and 0.2 µg of ghost

TABLE I
Quantitation of Rat Adipose Cell Glucose Transporter by Antibody and Cytochalasin B Binding

Levels of glucose transporter in membrane fractions from rat adipose cells were quantitated by scanning the autoradiogram shown in Fig. 1 as described in experimental procedures. Column 1 gives the result relative to the levels in basal microsomes. Column 2 gives the amount of D-glucose-inhibitable cytochalasin B binding, and column 3 gives the cytochalasin B binding relative to the binding by basal microsomes.

Membrane Fraction	Relative Amount of Glucose Transporter by Antibody	Cytochalasin B Binding Sites pmol/mg	Relative Cytochalasin B Binding Sites
Plasma Membranes: basal	0.59	8.5	0.09
insulin-treated	0.74	36	0.40
Microsomes: basal	1.00	90	1.00
insulin-treated	0.29	35	0.39

protein were readily visualized, compared with 10 to 50 fold higher amounts used previously (9,10). Note that 10 to 60 µg of rat adipose cell membranes had to be used in Fig. 1 because of the lower content of glucose transporter. The nonspecific labeling effect is illustrated by the fact that the polypeptide standards in lane 5 of Fig. 2, loaded at 11 µg each, are visible on the autoradiogram.

Quantitation of Labeling. Fig. 1 also demonstrates that the amount of labeling of the major adipose cell band varies between the plasma and low-density microsomal membranes depending upon the metabolic state of the cells prior to homogenization. Scans of the autoradiograms were used to construct curves of band intensity versus protein applied to the gel. From these plots the relative amounts of the major band in the four samples were estimated (Table 1). The number of D-glucose-inhibitable cytochalasin B binding sites in the membranes is also shown in Table 1. The insulin-induced changes in antibody labeling are similar to the changes in the number of cytochalasin B binding sites although the degree of increase of labeling in the plasma membrane fraction caused by insulin was much less than the increase in binding sites. In other experiments where human erythrocyte ghosts were run on the same gel with the rat adipose cell membrane fractions quantitation showed about 1000-fold less transporter per mg of

protein in adipose cell basal plasma membranes than in erythrocyte ghosts. Erythrocyte ghosts bind about 400 pmol of cytochalasin B per mg protein (17), which is only 10-fold higher than plasma membranes from insulin-treated adipose cells and 50-fold higher than basal plasma membranes. Thus, there is a discrepancy between quantitation of the transporter with antibody and with cytochalasin B in the different cell types which may reflect only partial cross-reactivity. In addition the lack of exact agreement between the antibody and cytochalasin B methods may be caused by differences in plasma membrane and microsomal membrane composition which interfere with antibody binding, although this seems unlikely since the polypeptides are separated on SDS gels before the antibody is added. It is also possible that there are different forms of the transporter. For example the affinity of the adipose cell plasma membrane transporter for cytochalasin B is significantly higher than that of the microsome fraction (18), and antibody cross-reactivity might also depend on specific forms of the molecule.

Identity of the Adipose Cell Glucose Transporter. The results suggest that the adipose cell glucose transporter is a protein with an apparent molecular weight of about 45,000. This conclusion is supported by the qualitative agreement between changes in the antibody-labeled band and number of D-glucose-inhibitable cytochalasin B binding sites in two membrane fractions in response to insulin. The similarity of the molecular weight of the labeled band to that of the human erythrocyte glucose transporter (46,000 after removal of most of the carbohydrate (16)) also supports this conclusion. However, the possibility of a cross-reacting protein unrelated to the glucose transporter, which fortuitously has a molecular weight similar to that of the erythrocyte transporter and changes its membrane distribution in a similar way in response to insulin has not been ruled out entirely. Experiments designed to provide more conclusive evidence of the identity of the adipose cell glucose transporter are in progress.

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