Potential Mechanism of Insulin Action on Glucose Transport in the Isolated Rat Adipose Cell

APPARENT TRANSLLOCATION OF INTRACELLULAR TRANSPORT SYSTEMS TO THE PLASMA MEMBRANE

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Detailed studies of [3H]cytochalasin B-binding and the inhibition of glucose transport by cytochalasin B have recently permitted characterization of a specific class of D-glucose-inhibitable cytochalasin B-binding sites in purified isolated rat adipose cell plasma membranes, identification of these sites as functional glucose transport systems, and direct demonstration that insulin stimulates glucose transport in intact cells primarily by increasing the number of these systems. An abbreviated [3H]cytochalasin B-binding assay is used here to examine the microsomal membrane fraction for D-glucose-inhibitable binding sites which could potentially represent those transport systems appearing in the plasma membranes in response to insulin.

In a series of three experiments, membranes in the microsomal fraction prepared from cells which have been preincubated for 30 min in the absence of insulin contain 38 pmol of D-glucose-inhibitable cytochalasin B-binding sites per mg of membrane protein, or approximately 5-fold that observed in the plasma membrane fraction prepared from the same cells. However, in the presence of 7.0 nM (1000 microunits/ml) insulin during the preincubation period, the number of these sites in the microsomal membrane fraction is decreased to 21 pmol/mg of membrane protein, while that in the plasma membrane fraction is increased to 24 pmol/mg of membrane protein.

The numbers of D-glucose-inhibitable cytochalasin B-binding sites in the intact cell have been estimated from the 5'-nucleotidase and NADH-cytochrome c reductase specific activities of each membrane fraction and the original homogenates. Of a total of approximately 3.16 × 10⁸ binding sites/cell in the basal cells, the plasma and microsomal membranes account for 0.35 × 10⁶ and 2.81 × 10⁶ binding sites/cell, respectively. While insulin does not alter the estimated total number of binding sites, that in the plasma membranes rises to 2.08 × 10⁶ sites/cell (a net increase of 1.73 × 10⁶ sites/cell) while that in the microsomal membranes decreases to 1.09 × 10⁶ sites/cell (a net decrease of 1.72 × 10⁶ sites/cell). The stoichiometric nature of this relationship further suggests that the increased numbers of glucose transport systems observed in the plasma membranes in response to insulin originate in an unidentified intracellular membrane pool associated with the microsomal membranes and that this apparent translocation process could potentially represent the mechanism of insulin's acute stimulatory action on glucose transport in the adipose cell.

A primary effect of insulin on adipose and muscle cell function is its stimulation of glucose transport activity (1-4). The mechanism of insulin's stimulatory action on glucose transport is, however, unknown. Glucose is transported across the cell's plasma membrane by a facilitated diffusion process in both the basal and insulin-stimulated states (5). While kinetic studies almost uniformly support the concept that insulin stimulates transport by increasing the maximum velocity (Vmax), and not the Michaelis-Menten constant (Km), of the transport system, kinetics alone cannot satisfactorily distinguish between an effect of insulin on the mobility of a fixed number of transport systems or on the number of these systems (6, 7). In spite of the continuing appearance of new reports on the kinetic approach to resolving this question (8-11), only the development of a method for quantitating the number of glucose transport systems can permit an unambiguous examination of the mechanism of the transport response to insulin (12).

In a recent report from this laboratory, such a method has been described (13, 14). Cytochalasin B, a potent inhibitor of glucose transport, binds to plasma membranes prepared from isolated rat adipose cells. This binding is extremely rapid, even at 4°C, and completely reversible. While this binding is saturable, it is complex. Curve-fitting analysis of this binding suggests that cytochalasin B interacts with multiple classes of binding sites. Nevertheless, a specific class of sites, representing only a small fraction of the total, can be identified by the competitive inhibition of binding to these sites by D- but not by L-glucose. The inhibition constant (Ki) of D-glucose for cytochalasin B-binding to these sites is approximately 35 mm, similar to that observed for the glucose transport system in the human erythrocyte (15), and complete inhibition of binding is achieved only in the presence of D-glucose concentrations on the order of 500 mm. Further, cytochalasin B-binding to the remaining classes of sites can be markedly inhibited by cytochalasin E, an analogue of cytochalasin B which does not inhibit transport activity, thus substantially improving the precision with which the number of D-glucose-inhibitable sites and their dissociation constant (Ki) can be determined. Fi-
nally, the $K_i$ of cytochalasin B for glucose transport itself in the plasma membranes is virtually identical with the $K_i$ for cytochalasin B-binding to the D-glucose-inhibitable class of binding sites. On the basis of these results, this class of sites has been identified as the glucose transport system and the number of such sites, a direct reflection of the number of functional transport systems. Furthermore, since the $K_i$ of D-glucose for cytochalasin B-binding to these sites is well above the 5 to 10 mM $K_m$ for glucose transport in intact cells (11, 16), a simple symmetrical model for the glucose transport process in adipose cells, where the $K_m$ for transport is equated with the constant for glucose binding to the transport system, no longer appears tenable.

When plasma membranes are prepared from isolated rat adipose cells which have been preincubated for 15 min in the presence of 7.0 mM (1000 microunits/ml) insulin, the number of D-glucose-inhibitable cytochalasin B-binding sites is increased 4- to 5-fold relative to that observed in plasma membranes prepared from basal cells (13, 14). Since the characteristics of the binding sites in the plasma membranes of the insulin-stimulated cells are virtually identical with those in the plasma membranes of the basal cells, these results suggest that insulin stimulates glucose transport in the adipocyte primarily by increasing the number of functional glucose transport systems. The present studies were undertaken to examine the microsomal membrane fraction of isolated rat adipose cells, treated as above, for D-glucose-inhibitable cytochalasin B-binding sites which could potentially represent those transport systems appearing in the plasma membranes in response to insulin.

**EXPERIMENTAL PROCEDURES**

For each experiment, adipose cells were isolated from the whole epidydimal fat pads of 36 to 64 ad libitum-fed (Purina Rat Chow, Ralston Purina Co.), 150- to 200-g male rats (CD strain, Charles River Breeding Laboratories) by the method described by Rodbell (17) and modified by Cushman (18). All incubation media were Krebs-Ringer bicarbonate (4/2-hydroxyethyl)-piperazineethanesulfonic acid (Sigma Chemical Co.) pH 7.4, containing 10 mM of untreated bovine serum albumin per ml (Bovine Serum Albumin Powder, Fraction V, Reheis Chemical Co.). Following isolation, the pooled adipose cells were distributed in 5-ml volumes to 25-ml polyethylene scintillation vials containing 7 ml of incubation medium (20 mM Tris-HCl, 1 mM EDTA, 255 mM sucrose, 0 or 7.0 mM insulin. All steps following homogenization were carried out at 4°C. Enough insulin (crystalline zinc insulin, courtesy of Dr. Ronald E. Chance, Eli Lilly Co.) to achieve final concentrations of 0 or 7.0 mM, and incubated for 30 min at 37°C.

Plasma and microsomal membrane fractions were prepared from the incubated cells by the differential centrifugation method described by McKeel and Jarett (19) and modified as follows. Incubated cells were washed twice and homogenized at 24°C in a buffer containing 20 mM Tris-HCl, 1 mM EDTA, 50 mM sucrose, and enough insulin (crystalline zinc insulin, courtesy of Dr. Ronald E. Chance, Eli Lilly Co.) to achieve final concentrations of 0 or 7.0 mM, and incubated for 30 min at 37°C.

Plasma and microsomal membrane fractions were prepared from the incubated cells by the differential centrifugation method described by McKeel and Jarett (19) and modified as follows. Incubated cells were washed twice and homogenized at 24°C in a buffer containing 20 mM Tris-HCl, 1 mM EDTA, 50 mM sucrose, and 0 or 7.0 mM insulin. All steps following homogenization were carried out at 4°C with this same buffer, but in the absence of insulin. Each homogenate was centrifuged at 16,000 X g for 15 min. The supernatant was aspirated and saved for preparation of the microsomal membrane fraction, the fat cake was discarded, and the pellet (containing plasma membranes, mitochondria, nuclei, and broken cells) was washed once and resuspended for preparation of the plasma membrane fraction. The plasma membrane fraction was obtained by centrifugation at 25,000 X g for 60 min on a discontinuous 1.12 M sucrose gradient in 20 mM Tris-HCl, 1 mM EDTA. The plasma membrane band was washed twice by resuspension in buffer and centrifugation at 48,000 X g, and resuspended to a final concentration of 3 to 5 mg of protein/ml. The microsomal membrane fraction was obtained from the initial supernatant by centrifugation at 100,000 X g for 70 min. The pellet was washed once and resuspended to a final concentration of 5 to 7 mg of protein/ml. Equilibrium D-glucose-inhibitable cytochalasin B-binding to each fraction was then measured.

In order to assay D-glucose-inhibitable cytochalasin B-binding in multiple membrane samples, the original 15-point binding isotherm procedure (13, 14) has been simplified. First, cytochalasin B is routinely added to each assay. Cytochalasin E does not inhibit either D-glucose-inhibitable cytochalasin B-binding or glucose transport itself, but markedly suppresses cytochalasin B-binding to those other sites present in purified adipose cell plasma membranes which account for up to 95% of the total cytochalasin B-binding (13, 14). In the presence of cytochalasin E, cytochalasin B binds almost exclusively to the D-glucose-inhibitable site. Second, the number of cytochalasin B concentrations used in each assay has been reduced from 15, covering a broad range, to 4, representing concentrations at which roughly 20, 40, and 60% of the D-glucose-inhibitable binding sites are occupied plus one very high concentration yielding an estimate of the unresolvable binding component (13, 14). In the presence of cytochalasin E, 4-point cytochalasin B-binding isotherms are sufficient for estimating both the number of D-glucose-inhibitable binding sites present and their $K_i$. Third, the binding assay buffer itself has been changed from one comprising 10 mM KH$_2$PO$_4$, 100 mM KCl, and 500 mM L-glucose, to that used throughout the fractionation procedure (13, 14). The presence of 255 mM sucrose in the latter buffer eliminates the necessity of using t-glucose to correct for the nonspecific effects of such high sugar concentrations.

The final, simplified cytochalasin B-binding assay is carried out as follows. Initially, 40 µl of a cytochalasin E (Aldrich Chemical Co.) solution in buffer containing 25% ethyl alcohol is added to 500 µl of membrane suspension and mixed. Then 250-µl samples are removed therefrom, added to 200 µl of buffer either containing or not containing D-glucose, and mixed again. Four 100-µl samples are removed from each of the two resulting suspensions and mixed with 20 µl of buffer containing increasing concentrations of [H]cytochalasin B (New England Nuclear and Aldrich Chemical Co.) and tracer [14C]sucrose (New England Nuclear). The final concentrations of ethyl alcohol, cytochalasin E, and D-glucose are, respectively, less than 1%, 2000 mM, and 500 mM. The external concentrations of cytochalasin B are approximately 40, 170, 420, and 20,000 nM. [14C]Sucrose is used to aid in determining trapped, unbound [H]cytochalasin B in the membrane pellets obtained during the binding assay and is, therefore, added in a constant proportion to the concentration of [H]cytochalasin B such that the final [14C] and [3H] counts per min in the mixture will be approximately the same.

Duplicate 50-µl samples of each of the eight final suspensions are then transferred to 175-µl centrifuge tubes, the membranes in each sample are pelleted by centrifugation, 25 µl of each supernatant is removed for measurement of the free [H]cytochalasin B concentration, and the remaining supernatant in each sample is aspirated and discarded. Each centrifuge tube with its pellet is transferred directly to a scintillation vial for measurement of bound [H]cytochalasin B. Glass centrifuge tubes are used in pelleting the plasma membrane fractions and are centrifuged at 48,000 X g for 20 min. Cellulose acetate centrifuge tubes are used in pelleting the microsomal membrane fractions and are centrifuged at 220,000 X g for 60 min. All scintillation counting is carried out in 5-ml polyethylene scintillation vials with 3.0 ml of scintillation fluid (Aquasol, New England Nuclear) containing 1.7% H$_2$O.

Procedure was determined by the method described by Lowry et al. (20) and modified by Peterson (21), using crystalline bovine serum albumin (Sigma Chemical Co.) as the standard. 5'-Nucleotidase and NADH-cytochrome c reductase activities were measured by the methods described by Avruch and Hoeld Wallach (22) and Dallner et al. (23), respectively. All calculations were carried out on the Dartmouth Time-Sharing System computer facilities.

**RESULTS AND DISCUSSION**

Three detailed experiments have been carried out. In each experiment, plasma and microsomal membrane fractions were prepared at the same time from the same homogenates of isolated rat adipose cells that had been preincubated for 30 min at 37°C in either the absence or presence of 7.0 mM (1000 microunits/ml) insulin. All preincubated cells in a given experiment came from a single pooled suspension of isolated cells prepared from a single large group of rats. The plasma membrane fraction, as defined here, comprises those membranes which were first sedimented at 16,000 X g in 15 min, then further purified by centrifugation at 23,000 X g for 60 min in a discontinuous 1.12 M sucrose gradient. The microsomal membrane fraction comprises those membranes which
remained in suspension when centrifuged at 16,000 × g for 15 min, but sedimented at 160,000 × g in 70 min.

Fig. 1 illustrates the Scatchard analyses (24, 25) of equilibrium [3H]cytochalasin B-binding to these two membrane fractions in the presence of 2000 nM cytochalasin E and in the absence or presence of 500 mM D-glucose for one representative experiment. As has previously been observed with 15-point binding curves (13, 14) and is illustrated here with 4-point binding curves, preincubation of cells with insulin markedly enhances cytochalasin B-binding in the absence of D-glucose to the plasma membrane fraction relative to that observed when cells are preincubated in the basal state (Fig. 1A). On the other hand, 500 mM D-glucose inhibits cytochalasin B-binding to both plasma membrane preparations such that practically identical curves are obtained. Radial axes in a Scatchard plot represent constant free cytochalasin B concentrations (24, 25). Subtraction of the curves obtained in the absence of D-glucose along these axes yields straight lines representing the D-glucose-inhibitable class of cytochalasin B-binding sites in the basal and insulin-stimulated states (Fig. 1B). In three experiments, insulin increased the average number of binding sites from 7.8 ± 1.7 to 24.4 ± 0.5 (mean ± S.E.) pmol/mg of plasma membrane protein, without significantly influencing this site's $K_d$ (168 ± 12 nM, mean ± S.E.).

In contrast, cytochalasin B-binding to the microsomal membrane fraction is generally somewhat greater than that to the plasma membrane fraction under all conditions (Fig. 1C).

Moreover, preincubation of cells with insulin reduces cytochalasin B-binding in the absence of D-glucose to the microsomal membrane fraction relative to that observed when cells were preincubated in the basal state. However, 500 mM D-glucose still inhibits cytochalasin B-binding to both microsomal membrane preparations such that practical identical curves are obtained. Furthermore, subtraction of the curves obtained in the presence of D-glucose from their respective curves obtained in the absence of D-glucose yields straight lines with slopes which are statistically indistinguishable from each other or from those observed when the plasma membrane fractions are assayed (Fig. 1D). In three experiments, insulin decreased the average number of these D-glucose-inhibitable cytochalasin B-binding sites (mean $K_d$ ± S.E. of 126 ± 10 nM) from 37.8 ± 3.8 to 21.2 ± 2.4 (mean ± S.E.) pmol/mg of microsomal membrane protein.

The experiments reported here have been carried out with adipose cells which were preincubated in the absence or presence of insulin for 30 min at 37°C. In a preliminary study of the time course of insulin action, preincubation of cells with insulin for approximately 30 s at 37°C increased the number of D-glucose-inhibitable cytochalasin B-binding sites in the plasma membrane fraction from 3.9 to 18.2 pmol/mg of membrane protein and decreased the number of these sites in the microsomal membrane fraction from 60.0 to 35.8 pmol/mg of membrane protein, values similar to those obtained with the 30-min preincubation. However, since the present methodology requires washing the preincubated cells at room temperature in homogenization buffer prior to cell disruption, a process consuming an additional 3 to 4 min, the true time course of these effects of insulin has not yet been determined. Nevertheless, the results presented above suggest that 1) the microsomal membrane fraction prepared from isolated rat adipose cells appears to contain glucose transport systems which are similar to those associated with the plasma membrane fraction; and 2) acute insulin pretreatment of intact cells reduces their numbers. The identity of these D-glucose-inhibitable cytochalasin B-binding sites in the microsomal membrane fraction with those in the plasma membrane fraction and with the glucose transport system itself remains to be confirmed. Detailed studies of both the binding of cytochalasin B and its inhibition of glucose transport, as previously carried out with purified plasma membranes (14), are still required.

The plasma and microsomal membrane fractions prepared here are neither homogeneous with respect to the species of membranes present therein nor recovered quantitatively from the homogenates of the preincubated cells (19). Therefore, in order to estimate the stoichiometry of the apparent reciprocal relationship in the number of D-glucose-inhibitable cytochalasin B-binding sites in these two membrane fractions in response to insulin, 5'-nucleotidase and NADH-cytochrome c reductase activities were measured in each membrane fraction.
Effects of insulin pretreatment of intact cells on the numbers of D-glucose-inhibitable cytochalasin B (CB)-binding sites in the plasma and microsomal membranes present in adipose cell homogenates prior to fractionation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Plasma membranes</th>
<th>Microsomal membranes</th>
<th>Homogenates</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Insulin</td>
<td>Basal</td>
</tr>
<tr>
<td>Total protein recovered (pmol/min/mg of protein)</td>
<td>55.6 ± 5.1</td>
<td>59.4 ± 6.8</td>
<td>48.9 ± 6.4</td>
</tr>
<tr>
<td>Measured specific CB-binding activity (pmol/mg of protein)</td>
<td>4.7 ± 1.0</td>
<td>14.7 ± 0.3</td>
<td>22.8 ± 2.3</td>
</tr>
<tr>
<td>ADP-ribosylation specific activity (pmol/min/mg of protein)</td>
<td>1.9 ± 0.21</td>
<td>11.6 ± 0.19</td>
<td>2.02 ± 0.25</td>
</tr>
<tr>
<td>Adjusted specific CB-binding activity (pmol/mg of protein)</td>
<td>2.7 ± 1.7</td>
<td>14.8 ± 0.4</td>
<td>26.2 ± 1.7</td>
</tr>
<tr>
<td>Calculated membrane protein in original homogenates (mg/cell)</td>
<td>122 ± 6</td>
<td>142 ± 37</td>
<td>37 ± 0.5</td>
</tr>
<tr>
<td>Calculated specific CB-binding activity in original homogenates (sites/mg of protein)</td>
<td>122 ± 6</td>
<td>142 ± 37</td>
<td></td>
</tr>
<tr>
<td>Net effects of insulin on calculated specific CB-binding activity in original homogenates (sites/mg of protein)</td>
<td>+1.73 ± 0.49</td>
<td>-1.72 ± 0.62</td>
<td>+0.01 ± 0.40</td>
</tr>
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</table>

Results are the means ± S.E. of the individual values for each parameter obtained in each of three separate experiments.

1 Calculations were based on the adjusted specific cytochalasin B-binding activities and calculated membrane protein in the original homogenates.

and the original homogenates. The specific association of the former enzyme activity with the adipose cell plasma membrane has been established (26). However, while an enrichment of the latter enzyme activity in the microsomal membrane fraction of the adipose cell has suggested at least some specificity in the subcellular distribution of this activity, an additional, but lower, activity indigenous to the plasma membrane has not been ruled out (19). Indeed, highly purified rat liver plasma membranes and Golgi apparatus have recently been shown to contain NADH-cytochrome c reductase specific activities representing 10% and 25%, respectively, of that found in endoplasmic reticulum (27). The microsomal membrane fraction of adipose cells prepared here has not yet been characterized in detail with respect to its composition but probably contains, at a minimum, components of Golgi apparatus and both smooth and rough surfaced endoplasmic reticulum (18, 19). Collectively, these three microsomal membrane species, free of contaminating plasma membranes, comprise the microsomal membranes described below.

If assumptions are made that 1) the specific activity of any NADH-cytochrome c reductase activity which may be indigenous to the plasma membranes can be represented as a fraction of that of the combined membrane species comprising the microsomal membranes (e.g., 0.2); 2) the 5'-nucleotidase specific activity of the microsomal membranes is 0; and 3) the two membrane fractions prepared here contain only plasma and microsomal membranes (19), then equations 1 can be developed which permit an estimation of the yields of these membranes in each fraction and the number of D-glucose-inhibitable cytochalasin B-binding sites present in these membranes in the original homogenates. Furthermore, since the number of cells homogenized has also been estimated (28), the results can be expressed, in turn, on a per cell basis. Table I illustrates the average values obtained in three experiments. Over the six homogenates studied, three each from basal and insulin-stimulated cells, approximately equal starting quantities of plasma and microsomal membranes are calculated to have been present prior to fractionation. In addition, the actual yields of membrane protein in each fraction, prior to correction for cross-contamination, are quite similar, as a percentage of the original total homogenate protein, to those reported by McKeel and Jarett (19) and represent roughly 50% of the estimated starting quantities of each membrane species in the homogenates.

Based on these estimates, the total numbers of D-glucose-inhibitable cytochalasin B-binding sites present in the homogenates of cells which have been preincubated in the absence or presence of insulin are virtually identical. Moreover, insulin decreases the number of binding sites present in the microsomal membranes in the basal state by a quantity which is also virtually identical with the insulin-stimulated increase in the number of binding sites present in the plasma membranes.

The calculated quantities of plasma and microsomal membranes and their respective calculated numbers of D-glucose-inhibitable cytochalasin B-binding sites present in the original homogenates depend, however, upon the extent to which NADH-cytochrome c reductase activity is assumed to be indigenous to the plasma membranes. For example, when the plasma membranes are assumed to be free of indigenous NADH-cytochrome c reductase activity (a fractional specific activity of 0), then the estimated quantity of plasma membranes present in the homogenates is decreased by 20%, and the estimated quantity of microsomal membranes present in the homogenates is increased by 30%, relative to those estimated quantities obtained using a fractional specific activity of 0.2 (Table 1). The estimated numbers of D-glucose-inhibit-
able cytochalasin B-binding sites in the plasma membranes in the homogenates of the basal and insulin-stimulated cells are correspondingly reduced to \(-0.26 \times 10^2\) (a negative value) and \(1.80 \times 10^4/\text{cell}\), respectively, while those in the microsomal membranes are increased to \(3.44 \times 10^4\) and \(1.43 \times 10^5/\text{cell}\). Nevertheless, the stoichiometric nature of the relationship between the increase in the number of binding sites in the plasma membranes (+2.06 \(\times 10^4/\text{cell}\)) and the decrease in the number of binding sites in the microsomal membranes (-2.01 \(\times 10^4/\text{cell}\)) in response to insulin is unchanged, although the overall magnitude of the effect of insulin is somewhat increased. When the plasma membranes are assumed to have a maximal indigenous NADH-cytochrome c reductase activity (approximately 0.25), then the overall magnitude of the effect of insulin is somewhat decreased relative to that obtained using a fractional specific activity of 0.2 (Table I).

Assignment of a correct value to the indigenous fractional NADH-cytochrome c reductase activity of the plasma membranes must await detailed studies of the distribution of this enzyme similar to those recently carried out using rat liver membrane fractions (27). Indeed, recent preliminary experiments in this laboratory in which the microsomal membrane fraction described here has been further fractionated by differential centrifugation at 50,000 \(\times g\) for 20 min suggest that a low density membrane species containing a markedly increased number of D-glucose-inhibitable cytochalasin B-binding sites and a decreased NADH-cytochrome c reductase activity may be separable from a higher density membrane species with the opposite characteristic activities. However, until further studies are undertaken, the choice of a value for the fractional NADH-cytochrome c reductase activity of the plasma membranes relative to that of the combined microsomal membranes remains relatively arbitrary. The results obtained with an assumed fractional NADH-cytochrome c reductase activity in the plasma membranes of 0.2 have been chosen for presentation in Table I since fractional activities greater than roughly 0.25 yield negative values for the estimated number of microsomal membrane contamination of the plasma membrane fractions, while fractional activities lower than roughly 0.15 yield negative values for the estimated number of D-glucose-inhibitable cytochalasin B-binding sites in the plasma membranes present in the homogenates of basal cells.

The results presented here suggest that exposure of intact isolated rat adipose cells to insulin for 30 min at 37°C appears to bring about, through some as yet unknown mechanism, a stoichiometric translocation of glucose transport systems from an intracellular membrane pool fractionating with the microsomal membranes to the plasma membrane. The consequence of such an apparent translocation is a minimum increase of 3-fold in the number of transport systems available in the cell’s plasma membrane for the uptake of glucose. The order of magnitude of this effect correlates well with that of insulin’s stimulatory action on glucose transport activity and on glucose metabolism at low extracellular glucose concentrations in intact isolated rat adipose cells under similar experimental conditions (7, 14, 16). Preliminary studies of the time course of this effect of insulin also suggest that it occurs as rapidly as can be measured (less than 5 min) with the present methodology and could, therefore, potentially represent the mechanism of insulin’s acute stimulatory action on glucose transport.

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Note Added in Proof—Since the submission of the present report for publication, the authors have received a manuscript from K. Suzuki and T. Kono which describes experiments where subcellular fractions of basal and insulin-stimulated adipose cells have been prepared by sucrose density gradient centrifugation and directly assayed for cytochalasin B-sensitive glucose transport activity following reconstitution into liposomes. Two peaks of activity are identified in fractions which approximately correspond to those described here. Furthermore, both the relative magnitudes of these two peak activities under basal conditions and their reciprocal relationship during the response to insulin parallel those on D-glucose-inhibitable cytochalasin B-binding observed in the present investigation. This manuscript is to be published in the May, 1980, issue of Proc. Natl. Acad. Sci. U. S. A.

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