Effects of Insulin and Insulin-like Agents on the Glucose Transport System of Cultured Human Fibroblasts

PAULOS BERHANU AND JERROLD M. OLEFSKY

SUMMARY

We have studied the effects of insulin and insulin-like agents on glucose transport by cultured human fibroblast monolayers. Initial rates of glucose transport were determined by measurement of 2-deoxy-D-glucose uptake. At physiologic concentrations, insulin stimulates 2-deoxy-D-glucose transport (average of 50% over basal) with a half-maximally effective insulin concentration of 3.3 ± 0.9 ng/ml. This effect of insulin is rapid and is half-maximal at 10 min and becomes maximal by about 30 min. Kinetic analyses showed that insulin increased the transport V_{max} from 7.4 ± 0.9 nmol/min/10^6 cells to 11.0 ± 1.5 nmol/min/10^6 cells and had no effect on the K_m value (2.5 ± 0.3 mM). While glucose starvation led to a higher overall rate of 2-deoxy-D-glucose transport, the relative stimulation by insulin was the same as in non-glucose-starved cells. Insulin mimickers [insulin-like growth factor (IGF), anti-insulin receptor antibody, and concanavalin A] also stimulate 2-deoxy-D-glucose transport by human fibroblast monolayers in a dose-dependent manner and the maximal effects of IGF and anti-insulin receptor antibody were the same as that of insulin, while the maximal effect of concanavalin A was only 78% of that of insulin. The maximal effects of either insulin and IGF or insulin and anti-insulin receptor antibody were not additive, suggesting that these agents all act via the same glucose transport effector system in human fibroblasts.

In conclusion, human fibroblasts possess an insulin-sensitive glucose transport system that displays many of the characteristics common to other more well studied transport systems. Thus, cultured human fibroblasts can serve as an important model for physiologic studies of insulin action and glucose transport, and for studies of pathophysiologic abnormalities of these processes in cells obtained from patients with various disease states. DIABETES *30*: 523–529, June 1981.

From the Department of Medicine, University of Colorado Health Science Center, Division of Endocrinology, Denver, Colorado.

Address reprint requests to Jerrold M. Olefsky, M.D., University of Colorado Health Science Center, Department of Medicine, B151, 4200 East Ninth Avenue, Denver, Colorado 80262.

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Insulin exerts a variety of cellular actions by first binding to its specific receptors on the plasma membrane of responsive cells. One of its major biologic actions is to stimulate overall glucose metabolism and this effect is largely, but not exclusively, due to acceleration of glucose transport into cells of target tissues.[1–5] To study the role of insulin binding and/or action in different physiologic and pathologic states in man, a variety of cells such as erythrocytes,[6] lymphocytes,[7,8] monocytes,[9] and adipocytes[10,11] have been used. While very important information has been obtained from studies using these cells, it becomes difficult to draw conclusions regarding the role of any possible genetic defect(s) in insulin action, since such cells have to be studied shortly after removal from the patient and, therefore, are influenced by the in vivo milieu at the time they are obtained.

The cultured human fibroblast system provides an attractive alternative, since these cells are readily obtained from patients, can be grown in large quantities for several generations of culture, and can be studied under controlled conditions removed from the in vivo influences of the donor. For these reasons there has been considerable recent interest in using these cells for studies of insulin binding and/or action.[12–15] The initial rates of glucose uptake have been studied in cultured human fibroblast monolayers[16] and in detached and suspended cells,[17] and the results have demonstrated that these cells possess a saturable and stereospecific glucose transport system that is time, temperature, and concentration dependent and is also inhibited by cytochalasin B. Several studies have also demonstrated that insulin stimulates glucose uptake[18–20] and oxidation[21] in cultured human fibroblasts in a time- and concentration-dependent manner,[18,20] although very high (nonphysiologic) insulin concentrations were used in most of the studies.

The present study was initiated (1) to examine the acute effects of physiologic concentrations of insulin on the initial

rate of human fibroblast glucose transport, using the glucose analog 2-deoxy-D-glucose, and (2) to examine if, as in other cells, the glucose transport system of human fibroblasts responds to some of the known mimickers of insulin action.

MATERIALS AND METHODS

Materials. Eagle's minimal essential medium (MEM) with Earle's balanced salts, biotin, and trypsin were purchased from Grand Island Biological Company (Grand Island, New York). Fetal calf serum (FCS) and bovine serum albumin (BSA, fraction V) were purchased from Reheis Chemical Company (Phoenix, Arizona). 2-Deoxy-D-[1-^{3}H]-glucose (19.5 Ci/mmol) and L-[1-^{3}H]-glucose (10.7 Ci/mmol) were purchased from Amersham (Arlington Heights, Illinois) and New England Nuclear (Boston, Massachusetts), respectively. 2-Deoxy-D-glucose, D-glucose, L-glucose, 3-O-methyl-D-glucose, concanavalin A (con A), Tris (hydroxymethyl) aminomethane HCl (Tris), and N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Hepes) were purchased from Sigma (St. Louis, Missouri), phloretin from Biochemical Laboratories (Redondo Beach, California), and cytochalasin B from Calbiochem-Behring Corp. (La Jolla, California). Single component procine insulin was obtained through the courtesy of Dr. R. E. Chance, Eli Lilly and Company (Indianapolis, Indiana). Insulin-like growth factor (IGF) was the kind gift of Dr. Renee Humbel, Zurich, Switzerland.

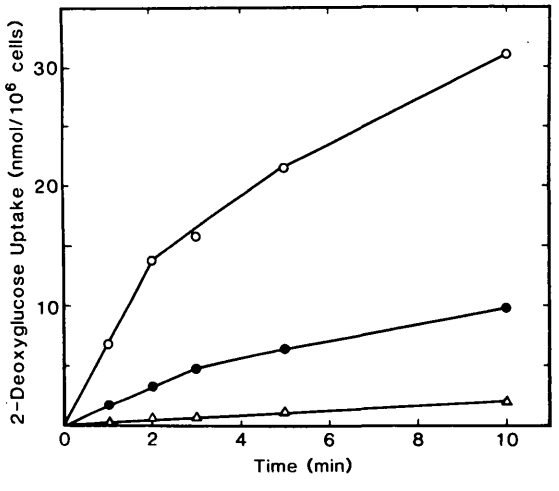
Cell culture. Normal human diploid fibroblasts obtained from forearm punch biopsy of skin were routinely grown as monolayer cultures in 75 cm^2 plastic T-flasks in antibiotic-free MEM supplemented with 26 mM $NaHCO_3$, 1 mg/L biotin, 10% (v/v) fetal calf serum, and were equilibrated with 5% CO_2 in humidified air at 37°C. Cell disaggregation for subculturing (once per week, approximately 2–3 days after visual confluency) was carried out using a solution of 0.5 mg/ml trypsin, 0.2% w/v EDTA in calcium, and magnesium-free Dulbecco's phosphate-buffered saline. Routine subculturing was performed at a 1:3 split and the cells were used for the experimental procedures between the 6th and 35th passages.

2-Deoxy-D-glucose transport studies. Measurement of the initial rates of hexose transport in human fibroblasts was carried out in triplicate on confluent cell monolayers grown in 60 × 15-mm plastic culture dishes after 8–10 days of growth, using 2-deoxy-D-glucose as the substrate. Each dish contained 5 ml MEM/10% FCS and the cells were not refed before the transport experiments. Under such culture conditions the cells were in a state of glucose starvation at the time of the transport studies, since glucose was consistently undetectable in the culture media by day 8 and the cells remained viable up to 2 wk without change of media (data not shown). We therefore elected to study 2-deoxy-D-glucose transport in the non-refed (glucose starved) state, since this minimized the number of culture manipulations and since it is also known that hexose starvation leads to a higher rate of glucose transport in fibroblasts.[20,22,23] Although we have not specifically examined the effect of serum starvation on hexose transport, it is likely that serum growth factors would also be depleted during the culture period before the transport studies. Before determinations of 2-deoxy-D-glucose transport each cell monolayer was rinsed twice with 3 ml of buffer (37°C, pH 7.45) containing 116 mM NaCl, 5.4 mM KCl, 1.0 mM $NaH_2PO_4 \cdot H_2O$, 2.0 mM Na_2HPO_4, 0.8 mM $MgSO_4 \cdot 7H_2O$, 1.0 mM $CaCl_2$, 0.2% BSA, and 25 mM Tris. The cells were then preincubated in this buffer for the desired periods of time at 37°C in a shaking water bath (60 oscillations/min) in a final volume of 1.8 ml with or without insulin or other agents. The transport reaction was initiated by the addition of ^{3}H-2-deoxy-D-glucose (0.4 μCi) and unlabeled 2-deoxy-D-glucose in a final volume of 200 μl. The reaction was terminated at the indicated time by rapid removal (vacuum aspiration) of the reaction media and washing each monlayer 4 times with ice-cold Krebs-Ringer bicarbonate (KRB) buffer containing 10 mM Hepes and 0.3 mM phloretin (pH 7.4); phloretin rapidly inhibits the transport process.[11,24] Then 1.0 ml of 1 N NaOH was added to each dish, and after rotating the dishes (100 oscillations/min for approximately 20–30 min, the solubilized cell material was transferred to scintillation vials. Each dish was then rinsed with 1.0 ml of 1 N HCl and the combined radioactivity determined in a liquid scintillation counter after addition of 15 ml of Aqueous Counting Scintillant (ACS, Amersham). Correction for diffusion and extracellular trapping of radioactivity was determined by subtracting the value for ^{3}H-L-glucose uptake measured in parallel with each 2-deoxy-D-glucose transport measurement (L-glucose rapidly equilibrates with the extracellular water space and enters cells only by simple diffusion[16,25]). In the experiments performed to estimate the K_m and V_{max} values, diffusion and extracellular trapping of 2-deoxy-D-glucose was assessed by measuring 2-deoxy-D-glucose uptake in the presence of 50 μM cytochalasin B, since cytochalasin B completely inhibits facilitated transport.[16,17,25–28] Cell counting after trypsinization of the replicate monolayers was carried out electronically using a Model ZB Coulter Counter with a 100-μm aperture, and ranged from 0.9–1.4 × 10^6 cells/60-mm dish.

RESULTS

Time course, temperature dependence, and specificity of the hexose transport system. The time course of 2-deoxy-D-glucose uptake (37°C) in the fibroblast monolayers at varying substrate concentrations (Figure 1) showed that

FIGURE 1. Time course of 2-deoxy-D-glucose uptake by human fibroblast monolayers at varying substrate concentrations. Uptake of 2-deoxy-D-glucose (37°C) was measured at the indicated times as described in MATERIALS AND METHODS at 2-deoxy-D-glucose concentrations of 0.1 mM (△), 1.0 mM (●), and 5.0 mM (○). Each point is the mean of three determinations.



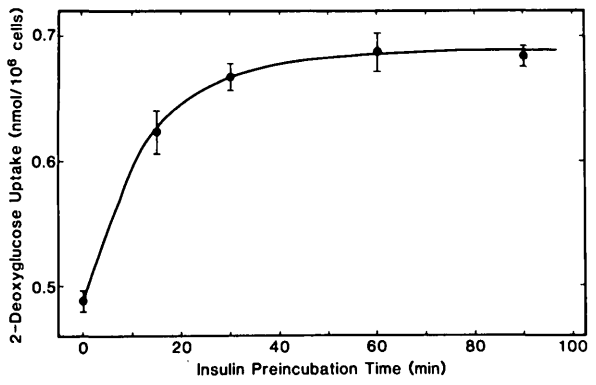


FIGURE 2. Time course of insulin action on 2-deoxy-D-glucose transport by human fibroblasts. Fibroblast monolayers were incubated at 37°C for the indicated time periods with 100 ng/ml of insulin. ^{3}H-2-deoxy-D-glucose transport (final concentration of 0.1 mM) was then measured over a 3-min period. The data represent the mean ± SEM of triplicate determinations from two experiments.

uptake was linear for up to 10 min at low (0.1 mM) 2-deoxy-D-glucose concentrations. However, at higher substrate concentrations, the duration of the initial period of linear uptake was progressively shortened (3 min and 2 min at 2-deoxy-D-glucose concentrations of 1.0 mM and 5.0 mM, respectively). Therefore, to insure the measurement of initial transport rates in all subsequent experiments, uptake was measured over a 3-min period at low 2-deoxy-D-glucose concentrations (≤0.1 mM) and over a 60–90-s period at higher 2-deoxy-D-glucose concentrations (>0.1 mM). The initial rate of 2-deoxy-D-glucose uptake at 37°C was 2.5-fold higher than the uptake at 21°C when uptake was measured over a 1–5-min period at a 2-deoxy-D-glucose concentration of 0.1 mM (data not shown).

Measurement of the initial rates of 2-deoxy-D-glucose uptake in the presence of 0.1–20 mM of unlabeled D-glucose, 3-O-methyl-D-glucose, or L-glucose showed that L-glucose had no effect on the uptake but D-glucose and 3-O-methyl-D-glucose competed for the uptake with half-maximal inhibitory concentrations of 0.5 mM and 8.0 mM, respectively (data not shown).

Cytochalasin B also rapidly inhibited 2-deoxy-D-glucose uptake in the fibroblasts with half-maximal inhibition occurring at a cytochalasin B concentration of 0.5 μM and maximal inhibition at 30–50 μM (data not shown). Additionally, 2-deoxy-D-glucose uptake in the presence of 50 μM cytochalasin B was equal to the value for L-glucose uptake, indicating that any apparent uptake of 2-deoxy-D-glucose in the presence of this concentration of cytochalasin B must represent diffusion or extracellular trapping of sugar.[3,16,17,25–29]

Effect of insulin on 2-deoxy-D-glucose transport. The time course of insulin action on 2-deoxy-D-glucose transport of human fibroblast monolayers is shown in Figure 2. Insulin rapidly stimulated 2-deoxy-D-glucose transport, and this effect became maximal (~50% stimulation over basal transport rate) by 30–60 min of incubation. All subsequent preincubations with insulin were for 60 min. Insulin activated 2-deoxy-D-glucose transport in a dose-dependent manner (Figure 3) and concentrations as low as 0.4 ng/ml (10 μU/ml) produced a measurable effect. Half-maximal stimulation occurred at an insulin concentration of 3.3 ± 0.9 ng/ml (mean ± SE), which is well within the physiologic concentration range. As has been observed previously in studies with cultured cells,[18,30] there was some variability in the absolute rate of 2-deoxy-D-glucose transport in different cell preparations. Nevertheless, comparable insulin dose-response curves were always observed, and the magnitude of stimulation by maximally effective concentrations of insulin (100 ng/ml) ranged from 17% to 60% (mean 50%).

When 2-deoxy-D-glucose transport was studied in glucose-starved cells and glucose-refed cells, the former had an overall higher rate of transport but the relative stimulation by insulin was essentially the same under both culture conditions (Table 1).

Figure 4 shows the effect of 2-deoxy-D-glucose concentration on sugar uptake both in the basal and in the maximally insulin-stimulated state. The nonsaturable (diffusion) component of uptake (as measured by uptake in the presence of 50 μM cytochalasin B) became a significant fraction of the total uptake at progressively higher 2-deoxy-D-glucose concentrations (Figure 4A). When the total uptake was corrected for this nonsaturable linear component by sub-

FIGURE 3. Effect of insulin concentration on 2-deoxy-D-glucose transport. Fibroblast monolayers were preincubated for 60 min at 37°C with the indicated concentrations of insulin. ^{3}H-2-deoxy-D-glucose transport (final concentration 0.1 mM) was then measured over a 3-min period. Each point is the mean ± SEM of triplicate determinations from three separate experiments.

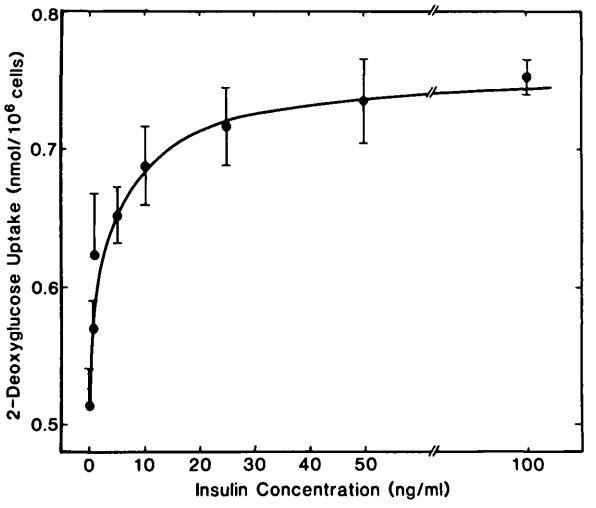


TABLE 1
Effect of glucose starvation on 2-deoxy-D-glucose transport

Preincubation condition	2-Deoxy-D-glucose transport (nmol/3 min/10⁶ cells)		% Stimulation by insulin
	Basal	+ Insulin	
+ Glucose (1 mg/ml)	0.99 ± 0.03	1.20 ± 0.06	21%
− Glucose	1.59 ± 0.02	1.88 ± 0.03	19%

Confluent monolayers of fibroblasts were preincubated for 24 h in growth medium lacking or containing D-glucose (1 mg/ml). At the end of the preincubation the cells were washed and incubated for an additional 60 min in the presence (100 ng/ml) or absence of insulin. 2-deoxy-D-glucose transport was then measured over a 3-min period as described in MATERIALS AND METHODS. The data represent the mean ± SEM of triplicate determinations from two separate experiments. The average cell density at the time of the transport studies was the same (0.91 × 10⁶ cells/dish) for both the glucose-refed and non-refed cells.

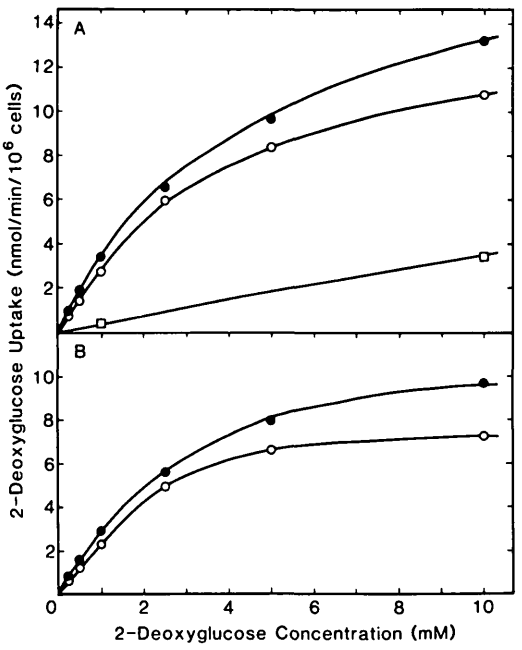
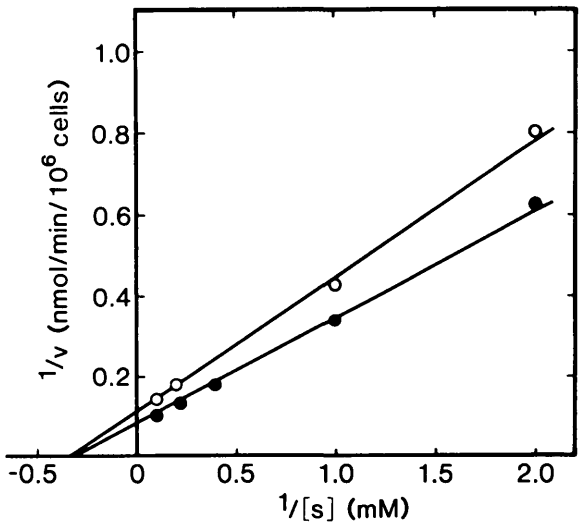


FIGURE 4. Effect of 2-deoxy-D-glucose concentration on sugar transport in the basal and maximally insulin-stimulated state. (A) Fibroblast monolayers were preincubated for 60 min at 37°C in the absence (○) or presence (●) of a maximally effective insulin concentration (100 ng/ml) and 2-deoxy-D-glucose uptake was then measured over a 1.5-min period at the indicated substrate concentrations. To assess the contribution of the diffusion component to overall transport, 2-deoxy-D-glucose uptake was also measured in the presence of 50 μM cytochalasin B (□), which inhibits all facilitated transport (see text). (B) Saturable facilitated transport in the basal (○) and insulin-stimulated (●) states after correcting the total uptake for diffusion. Calculations were made by subtracting the uptake in the presence of 50 μM cytochalasin B from the total uptake. The data represent the mean of triplicate determinations from three separate experiments.

tracting the uptake in the presence of 50 μM cytochalasin B from the total uptake, the resulting curves were hyperbolic and consistent with Michaelis-Menten Kinetics (Figure 4B). Analysis of the data using a Lineweaver-Burk plot (Figure 5) showed that insulin stimulated 2-deoxy-D-glucose transport by increasing the V_{max} from 7.4 ± 0.9 (mean ± SE)

FIGURE 5. Lineweaver-Burk plot of the corrected 2-deoxy-D-glucose transport data from Figure 4B for the basal (○) and insulin-stimulated (●) states.



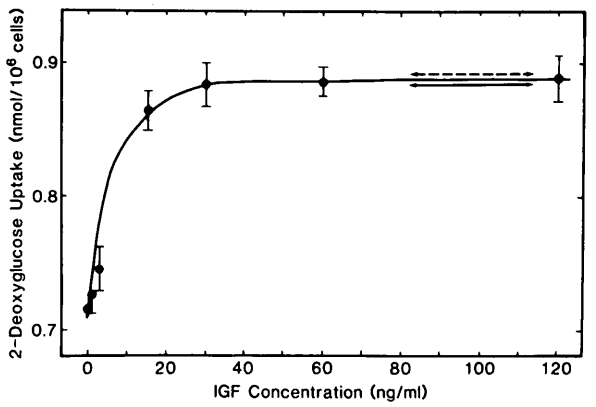


FIGURE 6. Effect of insulin-like growth factor (IGF) on 2-deoxy-D-glucose transport by human fibroblasts. Cell monolayers were preincubated for 60 min at 37°C with the indicated concentrations of IGF (●). 2-Deoxy-D-glucose transport (final concentration 0.1 mM) was then measured over a 3-min period. The horizontal solid arrow (←——→) indicates transport in the presence of 100 ng/ml insulin. The horizontal broken arrow (←——→) indicates transport in the simultaneous presence of 100 ng/ml insulin and 100 ng/ml IGF. All preincubations with the peptides were for 60 min. The data represent the mean ± SEM of triplicate determinations from two separate experiments.

nmol/min/10⁶ cells in the basal state to 11.0 ± 1.5 nmol/min/10⁶ cells in the maximally insulin-stimulated state. The K_m for 2-deoxy-D-glucose transport remained unchanged at 2.5 ± 0.3 mM in both the control and insulin-treated cells.

Effect of IGF, anti-insulin receptor antibody, and concanavalin A on 2-deoxy-D-glucose transport. Since insulin-like growth factor (IGF), lectins, and anti-insulin receptor antibody have been shown to mimick some of the actions of insulin,[12,27,31–35] we next investigated the effects of these agents on the glucose transport system of human fibroblasts. Figure 6 shows that IGF stimulates 2-deoxy-D-glucose transport in a dose-dependent manner, with half-maximal stimulation at an IGF concentration of 3.3 ng/ml. Insulin at a maximally effective concentration (100 ng/ml) gave the

FIGURE 7. Effect of concanavalin A on 2-deoxy-D-glucose transport by fibroblasts. Cells were preincubated for 60 min at 37°C with the indicated concentrations of concanavalin A (●) or with 100 ng/ml insulin. Transport was then measured over a 3-min period at a 2-deoxy-D-glucose concentration of 0.1 mM. The horizontal arrow (↔) indicates transport by cells preincubated with 100 ng/ml insulin for 60 min.

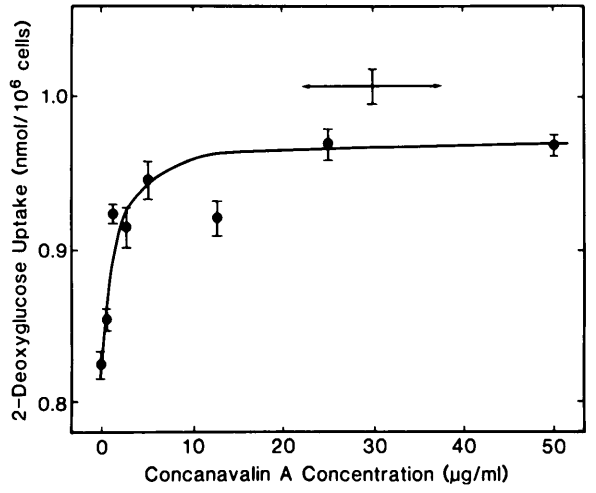


TABLE 2
Effect of insulin and anti-insulin receptor antibody on 2-deoxy-D-glucose transport

	2-Deoxy-D-glucose transport, % of control
Control fibroblasts	100
+ Insulin (100 ng/ml)	146 ± 7
+ Anti-insulin receptor antibody (1:20)	145 ± 9
+ Insulin (100 ng/ml) and anti-receptor antibody (1:20)	142 ± 8

Confluent monolayers of human fibroblasts were preincubated for 2 h at 37°C in the presence or absence of the indicated concentrations of insulin and/or anti-insulin receptor antibody. 2-Deoxy-D-glucose transport was then measured over a 3-min period as described in MATERIALS AND METHODS. The values represent the mean ± SEM of triplicate determinations.

same level of stimulation as that obtained with a maximally effective concentration of IGF. Moreover, when cells were incubated in the simultaneous presence of 100 ng/ml of insulin and IGF, the same maximal level of stimulation was observed as when either agent was used separately (Figure 6). This indicated that both insulin and IGF exerted their stimulatory effects on the same transport system, which has a fixed maximal response capacity.

Concanavalin A also stimulates 2-deoxy-D-glucose transport in a dose-dependent manner (Figure 7). Half-maximal stimulation occurred at con A concentration of 1 μg/ml with maximal stimulation at 25 μg/ml. The level of stimulation by maximally effective concentrations of con A (25–50 μg/ml) is approximately 78% of that obtained by 100 ng/ml of insulin, and this difference is statistically significant ($P < 0.05$).

The effect of anti-insulin receptor antibody isolated from the serum of a patient with acanthosis nigricans and insulin-resistant diabetes[33] was also studied. Preincubation of the fibroblast monolayers with a 1:20 dilution of this antibody resulted in a 45% increase in 2-deoxy-D-glucose transport, and no further increase was observed in the simultaneous presence of antibody (1:20 dilution) and 100 ng/ml of insulin (Table 2). A 1:20 dilution of this antibody has been previously shown to maximally inhibit insulin binding in cultured human fibroblast monolayers.[36]

DISCUSSION

Most previous studies of insulin action on glucose metabolism in cultured human fibroblasts have assessed its effects on glucose oxidation or glucose uptake and conversion into macromolecules.[12,19–21] Although there is a direct relationship between glucose transport and metabolism at low substrate concentrations, overall glucose metabolism involves multiple complex intracellular steps distal to the interaction of insulin with its cell surface receptors and, therefore, may be affected by other factors in addition to insulin. Thus, since the major initial action of insulin on glucose metabolism is the acute stimulation of glucose transport across the cell membrane,[37,38] we studied this process using physiologic concentrations of the hormone. The results obtained demonstrate several qualitative similarities to previous reports[20] of insulin's effect on human fibroblast glucose metabolism.

Initial characterization of our experimental system demonstrated that the cultured human fibroblasts possess a facilitated glucose transport system that is concentration-dependent, temperature-sensitive, stereospecific, and responsive to insulin and insulin-like agents. The characteristics of this transport system (time, concentration, and temperature dependence, stereospecificity, and inhibition by cytochalasin B) are in agreement with previous reports.[16,17] We chose 2-deoxy-D-glucose as the transport substrate since this hexose is transported by the glucose carrier system[29] and is phosphorylated but not further metabolized.[39] Furthermore, its time course of uptake at various substrate concentrations (Figure 1) was linear for long enough periods to allow the measurement of initial uptake rates. On the other hand, as reported previously by others,[20] preliminary experiments with the other widely used glucose analog, 3-O-methyl-D-glucose, indicated that its time course of uptake became rapidly curvilinear (10–15 s), thus making it technically difficult to obtain accurate measurements of initial uptake rates in fibroblast monolayers. Nevertheless, 2-deoxy-D-glucose has been used extensively as a substrate for transport studies and provides comparable results with 3-O-methyl-D-glucose.[40,41]

Insulin rapidly exerts its stimulatory effect on the human fibroblast 2-deoxy-D-glucose transport system and this effect is half-maximal at 10 min, 70% complete by 15 min, and becomes maximal by about 30 min of incubation (Figure 2). This effect, therefore, represents an acute activation of the existing transport system rather than an induction phenomenon.[29,42] The acute effect of insulin on the transport process is dose-dependent with half-maximal stimulation at an insulin concentration of 3.3 ± 0.9 ng/ml (5×10^{-10} M), which is well within the physiologic range. Our results on the time course and concentration dependence studies are qualitatively similar to those of Howard et al.[20] who studied insulin's effect on glucose uptake and incorporation into macromolecules. However, in their studies although measurable effects were observed at physiologic insulin concentrations, maximal effects were observed at insulin concentrations of 10^{-7} to 10^{-6} M and after 2–3 h of incubation.[20] In our previous study of human fibroblast monolayers,[36] half-maximal inhibition of insulin binding occurred at an insulin concentration of 7 ng/ml, which is about twofold higher than the half-maximally effective insulin concentration on 2-deoxy-D-glucose transport, thus indicating the presence of spare receptors.

As noted earlier we have observed variation in the absolute rate of 2-deoxy-D-glucose transport in different preparations of the cultured cells, but an insulin dose response was always observed. Moreover, when glucose-starved cells were compared with glucose-refed cells, the former had a significantly higher overall rate of 2-deoxy-D-glucose transport, but the percent stimulation by insulin was not significantly different from the non-starved cells (Table 1). The increase in the basal rate of transport in the glucose-starved cells is similar to previous findings in human and chick embryo fibroblasts.[20,22,23,43] However, in contrast to studies of insulin effect on D-glucose uptake and incorporation into glycogen and nucleotides,[20] where glucose starvation was necessary to observe insulin's stimulatory effect, we found that the acute activation of the initial transport rate of 2-deoxy-D-glucose by insulin does not require glucose starvation.

Kinetic analysis of the initial rates of 2-deoxy-D-glucose transport (Figures 4 and 5) indicate that insulin stimulates glucose transport in human fibroblasts by increasing V_{max} without changing the K_m. This finding is qualitatively analogous to previous reports of insulin's effect on the glucose transport system of human fibroblasts[18,20] and other cell types.[27,29] Thus, despite the fact that high nonphysiologic insulin concentrations and longer incubation periods were used,[18,20] we find comparable results using more physiologic insulin concentrations and shorter incubation periods. It is of interest to note that the V_{max} of transport in the basal state (7.4 ± 0.9 nmol/min/10^6 cells) is very similar to that of rat adipocytes.[29] However, while insulin causes a 300% or more increase in the V_{max} of 2-deoxy-D-glucose transport in the adipocyte,[29] the stimulation in fibroblasts is only about 50%. Thus, while the fibroblast glucose transport system and its mechanism of response to insulin are qualitatively very similar to that of a more traditional target cell for insulin action (i.e., the adipocyte), its main difference appears to lie in its limited capacity to respond to maximal stimulation by insulin.

Insulin-like growth factor (IGF),[12,31] anti-insulin receptor antibody,[32,33] and concanavalin A (con A)[34,35] mimick insulin action in other cell systems and also stimulate 2-deoxy-D-glucose transport in human fibroblast monolayers (Figures 6 and 7, Table 2) in a dose-dependent manner. Furthermore, the maximal effects of insulin, IGF, and anti-receptor antibody were comparable and not additive, suggesting that these agents all act via the same glucose transport system in human fibroblasts. It is interesting to note that fibroblasts are quite sensitive to the stimulatory effects of IGF on 2-deoxy-D-glucose uptake, with a half-maximal effect at an IGF concentration of 3.3 ng/ml. We[36] and others[31,44] have found that this concentration of IGF does not appreciably inhibit ^{125}I-insulin binding to fibroblasts, indicating that the effect of IGF to stimulate glucose transport is mediated through the IGF receptor rather than overlap of IGF into the insulin receptor.

In conclusion, these studies demonstrate that the human fibroblast glucose transport system shares similar qualitative features with those of other insulin target cells. These features include saturability, stereospecificity, inhibition by cytochalasin B, and activation by insulin and insulin-like agents. Since these cells represent a non-transformed human cell system, cultured fibroblasts can serve as an important model for studies of normal insulin action and glucose transport as well as investigations of genetic abnormalities of these processes.

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