Effect of Dexamethasone on Insulin Binding, Glucose Transport, and Glucose Oxidation of Isolated Rat Adipocytes

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ABSTRACT We have studied the in vitro effects of dexamethasone on isolated rat adipocytes at concentrations of dexamethasone therapeutically achieved in man. Glucose oxidation, glucose transport, and insulin binding were assessed. In dexamethasone-treated cells, glucose oxidation was decreased by 30-40% both in the absence of insulin (basal state) and at low insulin levels (less than 25 μU/ml). At maximally effective insulin levels (over 100 μU/ml) no differences existed between control and treated cells. If glucose transport were the rate-limiting step for glucose oxidation in the basal state and at low (submaximal) insulin levels, but not at maximally effective insulin concentrations, then these data could be explained by postulating that dexamethasone has a direct affect on glucose transport and does not affect intracellular oxidative pathways. We tested this hypothesis by directly assessing glucose transport in dexamethasone-treated cells. Glucose transport was assessed by measuring the uptake of [^{14}C]2-deoxy glucose. These studies demonstrated a 30-40% decrease in 2-deoxy glucose uptake by treated cells both in the basal state and at all insulin concentrations. Thus, a direct glucocorticoid effect on the glucose transport system seems to account for the decreased ability of dexamethasone-treated cells to oxidize glucose. Since dexamethasone treatment leads to decreased insulin binding to adipocytes in vivo, we examined the possibility that the in vitro decreases in insulin-mediated glucose transport could be due to decreased insulin receptors. Insulin binding to control and treated adipocytes was measured, and no differences were found. Therefore, in contrast to previously reported in vivo studies, adipocytes treated in vitro with dexamethasone retain a normal ability to bind insulin. Thus, these studies suggest that all of the in vitro effects of dexamethasone on glucose oxidation are due to direct inhibition of the glucose transport system.

Dr. Olefsky is a Research Associate with the Veterans Administration (MRIS #6488).

Received for publication 21 April 1975 and in revised form 7 August 1975.

INTRODUCTION

Insulin resistance, as manifested by glucose intolerance in the face of hyperinsulinemia, is a known sequela of in vivo corticosteroid administration (1, 2). It is well established in vitro that corticosteroids decrease the ability of isolated adipocytes (3-5) and muscle tissue (6) to oxidize glucose, and the potential relationship between these in vitro observations and in vivo insulin resistance is obvious. The initial studies of this in vitro effect of corticosteroids demonstrated that glucose oxidation could be inhibited in the absence of insulin (basal oxidation), but that this inhibition could be overcome by insulin (3-5). This led to the hypothesis that there were two distinct glucose transport systems: a basal system and an insulin-sensitive system, and that only the basal glucose transport system was sensitive to glucocorticoid inhibition (3-5). More recently, Czech and Fain (7) have reported that the inhibitory effects of dexamethasone on adipocyte glucose oxidation can only be observed where glucose transport is thought to be rate-limiting, and have therefore suggested that only one glucose transport system exists. To study this question, we have directly assessed glucose transport and oxidation in control and dexamethasone-treated cells.

We have previously demonstrated that in vivo administration of dexamethasone leads to a decrease in the number of insulin receptors on rat adipocytes and hepatocytes (8), and Kahn et al. (9) have reported similar results using rat liver plasma membranes. This led us to speculate that the dexamethasone-induced decrease in

insulin-mediated glucose metabolism (7, 10) might be due to decreased numbers of insulin receptors. To test this hypothesis, we studied the direct in vitro effects of dexamethasone on insulin binding to isolated adipocytes in conjunction with the measurements of glucose metabolism. Lastly, in these current studies, we sought to gain information about the interrelationships between insulin binding to receptors and insulin's cellular action (glucose transport and oxidation) in the same cells.

METHODS

Materials. Porcine monocomponent insulin was generously supplied by Dr. Ronald Chance of the Eli Lilly and Company (Indianapolis, Ind.). Na[^{125}I] was purchased from the New England Nuclear Co. (Boston, Mass.), bovine serum albumin (BSA)[1] (fraction V) from Armour Pharmaceutical Co. (Chicago, Ill.), collagenase from Worthington Biochemical Corp. (Freehold, N. J.), guinea pig anti-insulin antibody from Pentex Biochemical (Kankakee, Ill.). [1-^{14}C]Glucose, 2-deoxy [1-^{14}C]glucose, [^{14}C]inulin from New England Nuclear, and phlorizin and cytochalasin B from Gallard-Schlesinger Chemical Mfg. Corp., (Carle Place, N. Y.).

Preparation of isolated adipocytes. Male Sprague-Dawley rats were used for all experiments. All studies were performed in the morning on animals that had free access to standard rat chow. Animals were stunned by a blow to the head and decapitated, and epididymal fat pads were removed. Isolated fat cells were prepared by shaking at 37°C for 60 min in Krebs-Ringer bicarbonate buffer containing collagenase (3 mg/ml) and albumin (40 mg/ml), according to the method of Rodbell (11). Cells were then filtered through 250-μm nylon mesh, centrifuged at 400 rpm for 4 min, and washed three times in buffer (12).

Iodination of insulin. ^{125}I-insulin was prepared at a sp act of 100–150 μCi/μg according to Freychet and coworkers' modification (13) of the method of Hunter and Greenwood (14), as previously described (15). This preparation has an average of 0.2–0.3 atoms of ^{125}I/insulin molecule. Freychet (13) and others (16, 17) have demonstrated that with this degree of iodination, over 90% of the iodinated species is monoiodinated insulin, which retains full biological activity (13, 17).

Binding studies. Isolated fat cells were suspended in a buffer containing 35 mM Tris, 120 mM NaCl, 1.2 mM $MgSO_4$, 2.5 mM KCl, 10 mM glucose, 1 mM EDTA, and 1% BSA (18), pH 7.6, and incubated with ^{125}I-insulin and unlabeled insulin in siliconized 10-ml Erlenmeyer flasks in a 24°C shaking water bath as previously described (8, 12, 19). Methodologic studies (data not shown) have indicated that optimal steady-state binding conditions are achieved at 24°C after 45 min of incubation (12). The incubations were terminated as described by Gammeltoft and Gliemann (20) by removing 200-μl aliquots from the cell suspension and rapidly centrifuging the cells in plastic microtubes to which 100 μl of dinonyl phthalate oil had been added. Dinonyl phthlate has a specific gravity intermediate between buffer and cells, and therefore, after centrifugation, three layers resulted: cells on top, oil in the middle, and buffer on the bottom. The cells were then removed and the radioactivity was determined. All studies were done in triplicate.

In the dexamethasone studies, the glucocorticoid (8×10^{-8} M)[2] was added at the beginning of the 1-h collagenase digestion. After this, cells were preincubated for 90 min with or without dexamethasone before the binding studies were begun. Adipocyte counts were performed according to a modification of method III of Hirsch and Gallian (22), in which the cells were fixed in 2% osmium tetroxide in 0.05 M collidine buffer (made isotonic with saline) for 72 h at 37°C and then taken up in a known volume of 0.154 M NaCl for counting. Counting was performed on a Celluscope Model 112H particle counter with a 400 μm aperture (Particle Data, Inc., Elmhurst, Ill.). Adipocyte size was determined with a calibrated microscope according to the method of Di Girolamo et al. (23), and no differences in cell size were noted between control and dexamethasone-treated cells.

Glucose oxidation studies. The ability of adipocytes to oxidize glucose was determined according to the method of Rodbell (11). Adipocytes were incubated at 37°C with [1-^{14}C]glucose at a total glucose concentration of 2 mM in Krebs-Ringer bicarbonate buffer, pH 7.4, containing BSA (40 mg/ml) and dexamethasone (8×10^{-8} M). After 1 h of incubation, the generated $^{14}CO_2$ was collected and counted in a liquid scintillation counter. In the dexamethasone studies, the glucocorticoid was added at the beginning of the collagenase digestion. After the isolated cells were collected, they were incubated for an additional 90 min with 8×10^{-8} M dexamethasone before the oxidation studies. Thus, the total exposure of cells to dexamethasone before the beginning of the oxidation study was 2½ h. Control cells were treated in the same way except for the absence of dexamethasone.

Glucose transport studies. Transport studies were performed with the same cell centrifugation technique as described for the binding studies. Unless otherwise stated, isolated adipocytes were incubated with 2-deoxy-D-[1-^{14}C]-glucose (sp act 2 mCi/mM) at a concentration of 0.28 mM in Krebs-Ringer bicarbonate, pH 7.4, containing BSA (10 mg/ml) at 24°C. This assay measures the total uptake of the radiolabeled 2-deoxy-glucose and is based on the principle that while 2-deoxy-glucose is transported and phosphorylated by the same processes as D-glucose, it cannot be further metabolized (24). The assay is terminated by transferring 200-μl aliquots from the assay mixture to plastic microtubes containing 100 μl aliquots of dinonyl phthalate oil. The tubes are centrifuged for 30 s in a Beckman microfuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) and the assay is considered terminated when centrifugation begins. In experiments in which the stimulatory effect of insulin on uptake was measured, the cells were preincubated with insulin for 45 min at 24°C. The amount of sugar trapped in the extracellular water space of the cell layers was determined with [^{14}C]inulin according to the method of Gliemann et al. (25). Extracellular water space was measured in each experiment, and all data of sugar uptake were corrected for this factor. The percent of the total amount of sugar available that was trapped in the extracellular water space was a linear function of cell concentration, and averaged 0.033±0.001% at a concentration of 2×10^5 cells/ml. The amount of trapped sugar ranged from 2 to 10% of the total sugar uptake, depending on the conditions of incubation, and no differences existed between control and dexamethasone-treated adipocytes.

[1] *Abbreviation used in this paper:* BSA, bovine serum albumin.

[2] This concentration of dexamethasone approximates the level achieved in plasma after the intravenous injection of 0.5 mg dexamethasone in man (21).

The protocol for the 2-deoxy-glucose uptake studies was as follows: Dexamethasone (8×10^{-8} M) was added at the beginning of the 1-h collagenase digestion. After this, the cells were incubated with dexamethasone in Krebs-Ringer bicarbonate at 37°C for 45 min. Next the cells were incubated with or without the indicated insulin concentration for an additional 45 min at 24°C in the presence of the same concentration of dexamethasone. Thus, the total time of exposure to dexamethasone before the determination of sugar uptake was 2½ h. Untreated adipocytes were handled identically except for the absence of dexamethasone.

Hexokinase measurements. Hexokinase activity was assessed by measuring the amount of 2-deoxy-D-[1-^{14}C]glucose converted to a 2-deoxy glucose 6-phosphate according to the method described by Kletzien and Perdue (26).

RESULTS

Glucose oxidation studies. The effects of dexamethasone on glucose oxidation can be seen in Fig. 1. Basal glucose oxidation (in the absence of insulin) is decreased 30% in the dexamethasone-treated cells. In the presence of submaximal insulin concentrations, this decrease in $^{14}CO_2$ production by dexamethasone-treated cells is still present, but at a maximally effective insulin concentration, no difference in glucose oxidation between control and dexamethasone-treated cells was observed. Increasing the dexamethasone concentration 10-fold did not change these results, indicating that the data represent the maximal dose effect of the corticosteroid. Since the glucose concentration used in these studies was relatively low (2 mM), glucose transport is probably the rate-limiting step in the absence of and at low levels of insulin. On the other hand, at the maximally effective insulin concentration, the amount of glucose transported into the cell could be great enough to saturate the oxidation pathways. Thus, if dexamethasone exerted its effects at the level of glucose transport, and did not affect the intracellular oxidative processes, one could see decreased glucose oxidation at basal conditions and at submaximal insulin levels, with normal rates of glucose oxidation at maximal insulin concentrations.

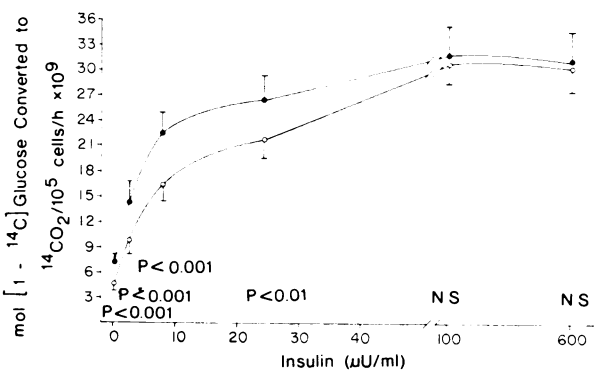

FIGURE 1 Ability of control (●—●) and dexamethasone-treated (○—○) adipocytes to oxidize glucose. Data represent the mean (±SE) of eight separate paired experiments. All P values were obtained by use of the paired t test.

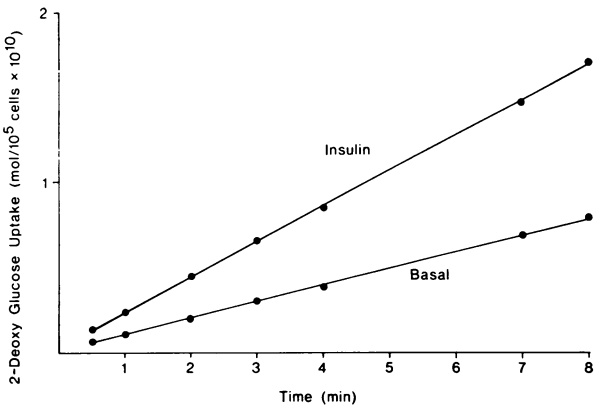

FIGURE 2 Time course of 2-deoxy-glucose uptake in the absence of (basal) and presence of insulin (5 ng/ml or 120 μU/ml). Incubations were carried out at 24°C in the presence of 0.28 mM 2-deoxy-glucose. Since 2-deoxy-glucose uptake is a linear function of cell concentration (not shown) up to at least 6×10^5 cells/ml (higher than used in any of the studies presented), all data are normalized to a cell concentration of 10^5 cell/ml.

2-Deoxy glucose uptake studies. To test this hypothesis, glucose transport was compared in control and dexamethasone-treated cells. Glucose transport was assessed by direct measurement of the rate of accumulation of the nonmetabolyzable sugar 2-deoxy-glucose, and to be confident that this approach allowed us to assess the glucose transport process, a number of initial studies were necessary.

Time-course experiments demonstrated that 2-deoxy-glucose uptake was linear until at least 8 min (Fig. 2) at 24°C, both in the absence of insulin and at maximally effective insulin concentrations. These experiments were conducted at a 2-deoxy-glucose concentration of 0.28 mM, and comparable linearity of uptake (until 5 min) was seen at 20 mM (data not shown). Therefore, all subsequent experiments were terminated at 3 min of incubation. As with other hexose transport systems, initial uptake rates were temperature-dependent (26-28), with 3-min 2-deoxy-glucose uptake being 50-70% greater at 37°C than at 24° (Table I). The sterospecificity of the system is shown by measuring the effect of other sugars on 2-deoxy-glucose uptake. This was done by incubating cells and 2-deoxy-glucose (1 mM) with other sugars such as L-glucose, D-fructose, 3-0-methyl-glucose, and phlorizin at a concentration of 10 mM. Under these conditions, 2-deoxy-glucose uptake was not affected by D-fructose or L-glucose, but was 57% inhibited by 3-0-methylglucose, 70% inhibited by D-glucose, and 80% inhibited by phlorizin.

The effect of substrate concentration on the initial rate

TABLE I

Effect of Temperature on 2-Deoxy-Glucose Uptake

Temperature	Insulin (5 ng/ml)	2-Deoxy-glucose uptake
°C		*nmol/10⁵ cells*
24	0	0.25
	+	0.52
37	0	0.39
	+	0.88

2-Deoxy-glucose concentration used was 0.125 mM and the data are expressed as the amount of sugar taken up by 10^5 cells at the end of 3 min. At 37°C uptake was linear until at least 5 min.

of uptake is seen in Fig. 3. The data show that uptake is a saturable function of 2-deoxy-glucose concentration below 5 mM, but that above this concentration initial uptake rates increase linearly with increases in 2-deoxy-glucose concentration. As with other hexose transport systems, the slope of uptake at the higher sugar concentrations (over 5 mM) is likely to represent simple inward diffusion of labeled substrate (26-29). This was confirmed by finding that in the presence of 50 μM cytochalasin B (which inhibits facilitated hexose transport [26-30]), a plot of initial influx rate versus 2-deoxy-glucose concentration gives a straight line with the same slope as that seen at the higher substrate concentrations in the absence of cytochalasin B. These data indicate that a saturable component of uptake exists in both the basal and insulin-stimulated state and that the transport process, as measured by this technique, behaves as a facilitated diffusion system (27-29, 31). Furthermore, when the data were corrected for the diffusion factor (26-29), and plotted on a Lineweaver-Burk graph (Fig. 3B), the apparent K_t of 2-deoxy glucose uptake was 2 mM in the basal and insulin-stimulated state, while the apparent V_{max} increased from 0.6 nmol/min/10^5 cells in the basal state to 1.8 nmol/min/10^5 cells in the insulin-stimulated state. Thus, in this system, insulin exerts its major effect on the apparent V_{max} of glucose uptake with little or no effect on K_t. This effect is similar to that reported by others (4), including Czech et al. (28), who used 3-0-methyl glucose to directly study glucose transport in brown fat cells. However, other workers have found insulin to effect primarily K_t (32), or both K_t and V_{max} (33) and the difficulty in reconciling these contrasting findings has been discussed (28).

To assess the intracellular fate of the 2-deoxy: [^{14}C]-glucose, we measured the percent of the intracellular radioactivity (Table II) in the form of free 2-deoxy glucose versus 2-deoxy glucose-6-phosphate, by the method of Tsuboi and Petricciani (34). The results of these studies show that the great majority of the labeled sugar was phosphorylated. Thus, since intracellular accumulation of free 2-deoxy: glucose did not occur at the lower concentrations of 2-deoxy-glucose (0.125-1 mM), and since the sugar concentration employed in all subsequent studies was 0.28 mM, the capacity of the hexokinase reaction is not exceeded, indicating that uptake of sugar closely reflects transport (26, 27, 35).

Since 2-deoxy glucose uptake is a measure of glucose transport, comparison of the data in Figs. 1 and 3 indicates that insulin has a greater effect to stimulate glucose oxidation than transport. One possible explanation is that insulin promotes glucose oxidation by affecting intracellular events, as well as by increasing glucose

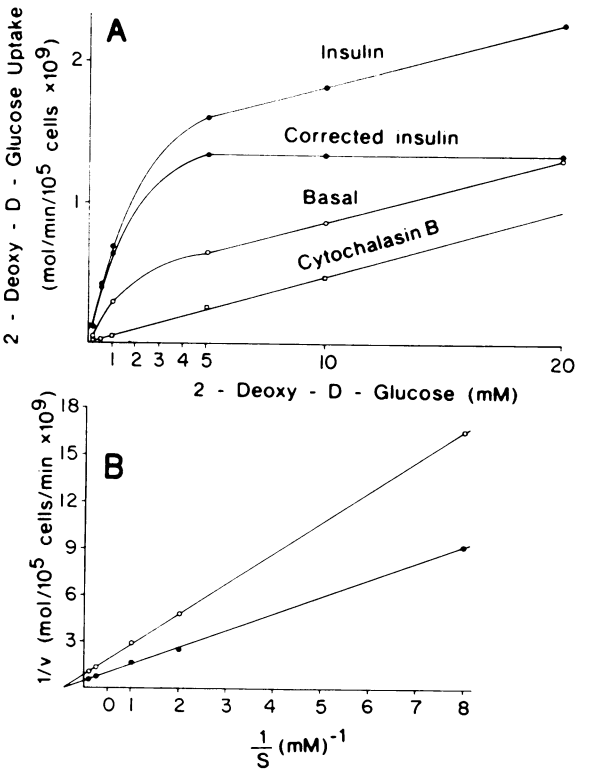

FIGURE 3 Effect of substrate concentration on 2-deoxy-glucose uptake. A. Adipocytes were incubated at the indicated sugar concentrations in the absence of insulin (basal, ○—○) and in the presence of 120 μU/ml insulin (insulin, ●—●). Parallel incubations were carried out identically except that cytochalasin B (50 μM) was added 20 min before measurement of sugar uptake (□—□). At 2-deoxy-glucose concentrations over 5 mM, the slopes of the basal, insulin-stimulated, and cytochalasin B data are the same, and represent simple inward diffusion of the sugar into the cells (26–29). Consequently, for kinetic analysis, the data are corrected by subtracting the amount of uptake in the presence of cytochalasin B from the total uptake in the absence of cytochalasin B (26–29) (corrected insulin ●—●). B. Lineweaver-Burk plot of the corrected basal (○—○) and insulin-stimulated (●—●) data of A above.

TABLE II

Distribution of Labeled Sugar between Free and Phosphorylated Products

Incubation 2-Deoxy-glucose concentration	2-Deoxy-glucose	2-Deoxy-glucose-6-phosphate
μM	*% of total*	
0.125	7	93
1	5	95
10	34	66

These studies were done according to the method of Tsuboi and Petricciani (34), in which the amount of intracellular 2-deoxy glucose present in the free and phosphorylated state is measured at the end of 3 min of incubation. Cell homogenates are fractionated on an ion-exchange column and the percent of the sugar that is free or phosphorylated is determined.

transport. Since this formulation is contrary to currently held views of insulin's action (36, 37), which maintain that insulin promotes glucose oxidation solely by promoting transport, experiments were designed to test this hypothesis. The data in Fig. 4 summarize these studies. When 2-deoxy-glucose uptake is measured in the presence of cytochalasin B, facilitated glucose transport is inhibited. Thus, the only glucose that enters the cell enters by simple diffusion. Since insulin does not affect this diffusion process (Fig. 3), 2-deoxy-glucose uptake in the presence of cytochalasin B is the same with or without insulin (Fig. 4A). In contrast, when glucose oxidation is measured under similar conditions (Fig. 4B), insulin exerts stimulatory effects even in the presence of cytochalasin B. In these experiments, high glucose concentrations were used, since at these concentrations appreciable amounts of sugar can diffuse into the cell (Fig. 3). Consequently, even though insulin cannot increase glucose transport in the presence of cytochalasin B, enough glucose enters the cell by diffusion so that a stimulatory effect distal to transport that promotes oxidation can be measured. The data of Fig. 4 are consistent with such an effect.

Comparison of 2-deoxy: glucose uptake in control versus dexamethasone-treated cells. Uptake of 2-deoxy-glucose is decreased in dexamethasone treated cells. These data are seen in Fig. 5, and support the hypothesis that dexamethasone exerts its effects at the level of glucose transport. Sugar uptake is decreased in the dexamethasone-treated cells in the absence of insulin (basal transport) and at all levels of insulin stimulation. Thus, uptake is 30-40% less by treated cells under all conditions, and this decrease is quantitatively comparable to the decreases in glucose oxidation noted in Fig. 1. However, if the data are expressed as the percent increase above the basal rate of uptake (Fig. 5B) the percentage

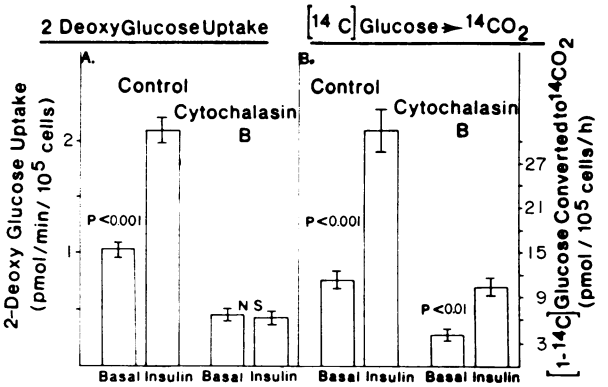

FIGURE 4 Effect of insulin (25 ng/ml or 600 μU/ml) on 2-deoxy-glucose uptake and glucose oxidation in the presence of cytochalasin B (50 μM). Adipocytes were preincubated with cytochalasin B as described in the legend to Fig. 3, and 2-deoxy-glucose uptake (A) or glucose oxidation (B) was measured in the basal state and in the presence of insulin. To maximize diffusion of glucose into the cell, high concentrations (10 mM) of hexose were used in these experiments. Data represent the mean±SE of four experiments.

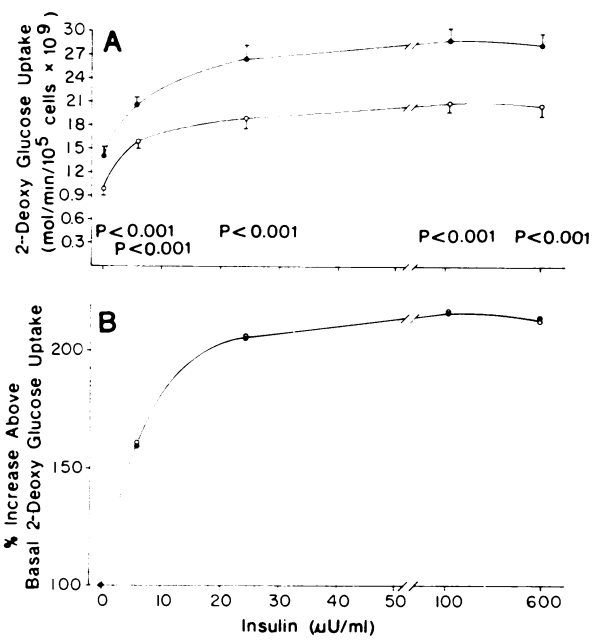

FIGURE 5 Ability of control (●—●) and dexamethasone-treated cells (○—○) to take up 2-deoxy-glucose. A. All cells were preincubated with or without insulin (at the indicated concentrations) for 45 min at 24°C. Uptake was then measured at the end of a 3-min incubation with [2-^{14}C]deoxy glucose (0.28 mM). Data represent the mean (±SE) of eight separate paired experiments. All P values were obtained by use of the paired t test. B. Data from control (●—●) and dexamethasone-treated (○—○) cells are plotted as percent increase above basal uptake values.

of increase in 2-deoxy-glucose uptake in the presence of insulin is the same for both groups of cells. Increasing the dexamethasone concentration 10-fold did not change these results. To see if the absolute decrease in 2-deoxy-glucose uptake demonstrated in Fig. 5A was due to a limited capacity of cells to transport the sugar, we incubated control and dexamethasone-treated cells with increasing 2-deoxy-glucose concentrations in the presence and absence of insulin (120 μU/ml). These results are seen in Fig. 6A. The data are similar to those in Fig. 3, with saturable and nonsaturable (diffusion) uptake components. At the higher sugar concentrations the slope of the nonsaturable uptake is the same for both groups of cells, and the corrected curves are seen in Fig. 6B. From these data it is clear that dexamethasone-treated cells are capable of taking up much larger amounts of 2-deoxy-glucose than observed at the low substrate concentrations (0.28 mM) used in Fig. 5. Thus the decrease in 2-deoxy-glucose uptake seen in Fig. 5 is not due to saturation of the transport system. Furthermore, when the data of Fig. 6B are plotted on a Lineweaver-Burk graph (not shown) dexamethasone-treated cells have the same apparent K_m as control cells (2 mM) but have a lower apparent V_{max}, and insulin increases the V_{max} with no appreciable effect on the K_m in both groups of cells.

Finally, since the slopes of the relationship between uptake and 2-deoxy glucose concentrations greater than 5 mM are the same for control and dexamethasone-treated cells, Fig. 6A indicates that dexamethasone does not affect the diffusion component of the glucose transport system.

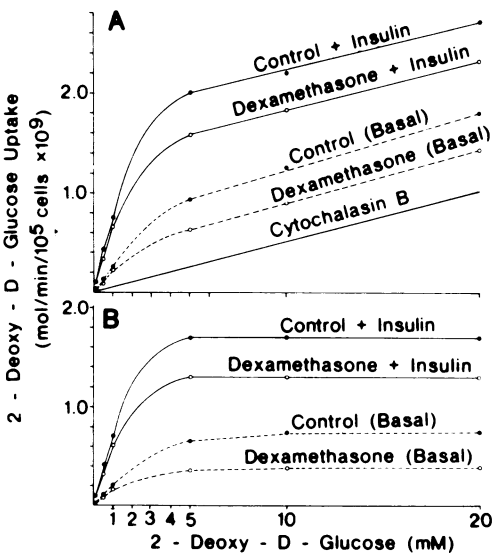

FIGURE 6 A. Effect of substrate concentration on 2-deoxy glucose uptake by control (●) and dexamethasone-treated cells (○) in the basal state (- - - - -), in the insulin-stimulated (120 μU/ml) state (——), and in the presence of 50 μM cytochalasin B (——). Plots represent the uncorrected data for all groups of cells. B. Data from A (above) after correction for inward diffusion component (see legend to Fig. 3).

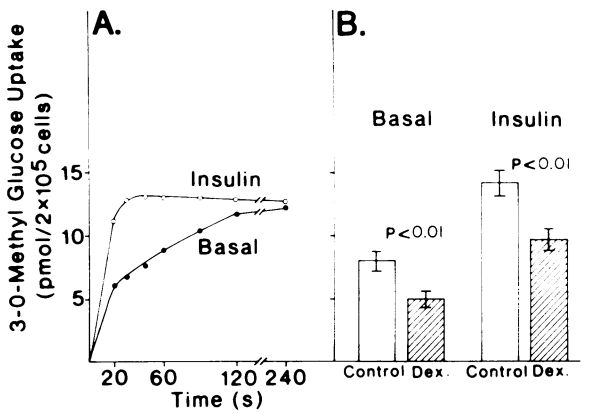

FIGURE 7 Inhibition of basal and insulin-stimulated 3-O-methyl glucose uptake by dexamethasone. A. The time course of 3-O-methyl glucose uptake in the absence (●—●) and presence (○—○) of insulin (5 ng/ml or 120 μU/ml). The 3-O-methyl glucose concentration was 20 μM, and uptake was assessed as described for the 2-deoxy-glucose studies. B. The effects of dexamethasone (shaded bars) on 30-s 3-O-methyl glucose uptake. Data represent the mean ±SE of three experiments.

From the above data, several lines of reasoning support the contention that dexamethasone inhibits the glucose transport system (see Discussion). However, additional studies were done assessing the uptake of 3-O-methyl glucose to further corroborate this conclusion. The results of these experiments are seen in Fig. 7. Since this analog is not phosphorylated, its uptake is solely a measure of transport (28, 35, 38). Fig. 7A shows the uptake of 3-O-methyl glucose as a function of time. Because 3-O-methyl glucose is not phosphorylated (and consequently trapped inside the cell), equilibrium is rapidly reached due to significant efflux of the sugar. Thus, uptake is nonlinear, even in the basal state, by as early as 45 s, and in the presence of insulin, net uptake is at near equilibrium by 20 s. These results are comparable to other data of 3-O-methyl glucose uptake by isolated adipocytes (36, 39), and indicate that, with current techniques, this approach is not ideal for the study of glucose transport in these cells. Nevertheless, as seen in Fig. 7B, dexamethasone had consistently decreased both basal and insulin-mediated 3-O-methyl glucose uptake in these cells. These observations strongly support the results of the 2-deoxy : glucose studies.

Lastly, to be certain that dexamethasone did not affect the hexokinase reaction during this relatively short in vitro exposure to the glucocorticoid, total hexokinase

activity was measured in control and dexamethasone-treated cells. Using the method of Kletzien and Perdue (26; see Methods) no differences in hexokinase activity were found (data not shown).

Insulin binding studies. Since we have previously demonstrated that dexamethasone in vivo will decrease insulin binding to isolated adipocytes (8), it seemed possible to speculate that the decrease in insulin-mediated glucose oxidation and transport just described was due to a dexamethasone-induced decrease in insulin binding. To study this possibility, insulin binding was determined with control and dexamethasone-treated cells, and the results of these studies are presented in Fig. 8. As can be seen, the ability of both groups of cells to bind insulin is identical, indicating that under these experimental conditions dexamethasone does not affect insulin binding in vitro. The concentration of dexamethasone used in these experiments was 8×10^{-8} M, which should be fully effective. However, increasing the dexamethasone concentration 10-fold or prolonging the preincubation period with the glucocorticoid to 5 h did not change these results.

Since dexamethasone did not decrease insulin binding (Fig. 8), but since dexamethasone did decrease glucose transport (Fig. 5A), one can see that for any given amount of insulin bound per cell, the dexamethasone-treated adipocytes will transport less glucose. However,

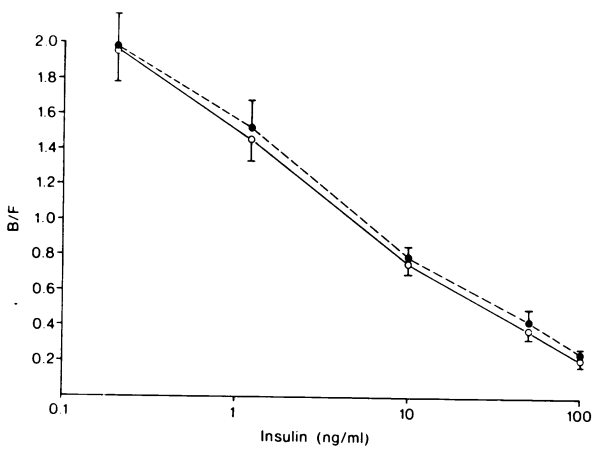

FIGURE 8 Ability of control (●—●) and dexamethasone-treated cells (○—○) to specifically bind ^{125}I-insulin. Cells were incubated for 45 min at 24°C with 3.3×10^{-11} M ^{125}I-insulin plus unlabeled insulin to give the indicated total insulin concentrations. B/F represents the ratio of bound to free ^{125}I-insulin. Data represent the mean (±SE) of six separate paired experiments and all data were corrected for nonspecific binding by subtracting the amount of radioactivity remaining bound at an insulin concentration of 200 μg/ml from the amount of radioactivity in the cell pellet at all other insulin concentrations (8, 12, 19). In these studies nonspecific binding averaged 4–8% of the total amount bound.

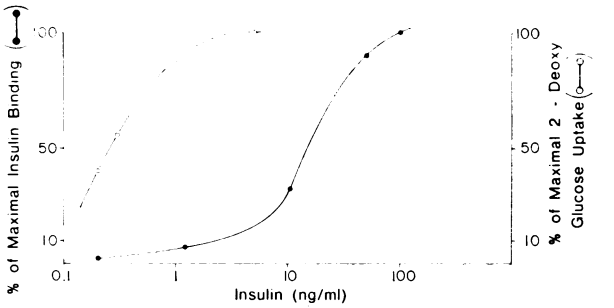

FIGURE 9 Relationship between dose-response curves of insulin binding (●—●) and 2-deoxy-glucose uptake (○—○). Open circles represent the percent of maximal insulin-mediated sugar uptake at the indicated insulin concentrations. Since Fig. 4B demonstrates that the percent increment above basal uptake is the same for control and dexamethasone-treated cells, this curve is also the same for both groups of cells. Closed circles represent the percent of maximal specific insulin binding for both groups of cells. For this analysis, maximal insulin binding is taken as the amount of insulin specifically bound at a total insulin concentration of 100 ng/ml (or 2,400 μU/ml) (12).

since the insulin-mediated increment in glucose transport above basal rates was the same in both groups of cells (Fig. 5B), it is probable that the interaction between insulin-receptor complexes and the glucose transport system is unaffected by dexamethasone. In both groups of cells, however, it is clear that increases in insulin concentration lead to increases in the amount of insulin bound far above the insulin concentration that causes maximal glucose transport. The relationship between the dose-response curves for insulin binding and glucose transport is seen in Fig. 9. These data show that maximal insulin-stimulated glucose transport occurs when only 15% of the available insulin receptors are occupied.

Mechanism of the dexamethasone effect. Glucocorticoids are known to initiate their effects by specifically binding to cytoplasmic receptors (40). Consistent with this mechanism is the 1-2-h time lag before effects of dexamethasone on glucose metabolism can be observed (4, 5, and unpublished observations). Furthermore, we have found that 8×10^{-8} M progesterone did not inhibit glucose oxidation (Table III), indicating that the observed effects of dexamethasone at this concentration were not due to nonspecific steroid-membrane interactions. Additionally, when adipocytes were exposed to dexamethasone (8×10^{-8} M) in the presence of excess amounts of progesterone (10^{-6} M), no effect of dexamethasone on glucose transport was observed (Table III). This indicates that the excess progesterone competitively inhibits the binding of dexamethasone to its receptor, and is consistent with progesterone's known properties as an inactive glucocorticoid receptor agonist

TABLE III

Effects of Progesterone on Glucose Oxidation

Addition	Glucose oxidation	
	Basal	Insulin (8 μU/ml)
	nmol/h	
None	8.1	26.7
Dexamethasone (8×10^{-8} M)	4.1	15.3
Progesterone (8×10^{-8} M)	7.9	25.4
Dexamethasone (8×10^{-8} M) +progesterone (10^{-5} M)	7.9	27.6

Preincubations with steroids were carried out for 2½ h before measurement of sugar uptake. Data are expressed as nanomoles of glucose converted to CO_2 per 10^5 cells per hour, and as seen in Fig. 1, 8 μU/ml insulin is a submaximal insulin concentration.

(41). These findings indicate that dexamethasone initiates its effects on glucose transport by binding to a specific glucocorticoid receptor.

DISCUSSION

Several investigators have found that dexamethasone decreases the ability of adipocytes to oxidize glucose in the absence of insulin, but that dexamethasone does not decrease glucose oxidation in the presence of insulin (3-5). From these studies it has been suggested that there are two separate glucose transport systems, one sensitive to dexamethasone and the other to insulin (3-5). However, more recently, Czech and Fain (7) have shown that at low glucose levels and submaximal insulin concentrations, dexamethasone does inhibit oxidation and that this inhibitory effect can only be overcome at maximal doses of insulin. This led to the suggestion that if glucocorticoids exert their effect on glucose oxidation by affecting the glucose transport system, then these effects will only be observed where glucose transport is rate-determining for oxidation. In the studies in which dexamethasone did not inhibit insulin-stimulated glucose oxidation (3-5), high glucose concentrations and maximal doses of insulin were employed —conditions under which glucose transport may not be rate limiting. Our data are clearly comparable to those of Czech and Fain, since we find that dexamethasone inhibits glucose oxidation in the basal state and at submaximal insulin concentrations, but did not inhibit oxidation at maximal insulin levels. These data allow one to infer that in vitro dexamethasone does not affect the intracellular enzyme systems involved in glucose oxidation, and, thus, an inhibitory effect of dexamethasone on glucose oxidation will be observed only if glucose transport is not great enough to saturate these intracellular systems.

Direct experimental support for these inferences was obtained with the 2-deoxy-glucose uptake studies. In these experiments, inhibition of basal and insulin-stimulated sugar uptake by dexamethasone was demonstrated. These data clearly demonstrate that dexamethasone exerts its effect at the level of glucose transport. In Fig. 5B it can be seen that dexamethasone does not alter insulin-stimulated uptake if the data are expressed as the percent increase above basal. This indicates that the glucocorticoid inhibits a fixed proportion of the transport system, and that basal and insulin-stimulated glucose transport share the same system. Furthermore, the data in Table III indicate that this is a specific glucocorticoid effect, probably mediated through a specific glucocorticoid receptor.

The conclusions drawn from the studies of 2-deoxy-glucose uptake are valid only insomuch as this measurement reflects glucose transport, and the validity of this concept has been recently reviewed (38). For example, Renner et al. (27) have extensively studied the glucose transport system in rat hepatoma cells, and have concluded that "2-deoxy-glucose is the substrate of choice as a model for glucose transport" since it is phorphorylated by cells (but not further metabolized) and consequently is trapped inside the cell allowing for readily measurable periods of maximal uptake. That this is the case for adipocytes can be seen in the time-course data of Fig. 2. However, since 2-deoxy-glucose is phosphorylated, it is conceivable that the hexokinase reaction is rate limiting. If so, then uptake would reflect phosphorylation more closely than transport, and the differences we have found between control and dexamethasone-treated cells may be due to differences in hexokinase activity rather than transport. We beliver this is highly unlikely for the following reasons: (*a*) the effects of dexamethasone on 3-0-methyl glucose uptake (which reflects only transport) are the same as its effects on 2-deoxy-glucose uptake. (*b*) The K_m values we measured for glucose transport are comparable to those reported by other workers (26-28, 38), and the effect of insulin to increase V_{max} without changing K_m is the same as that found by Czech et al. (28), who studied glucose transport in isolated brown fat cells with 3-0-methyl glucose. (*c*) Cytochalasin B, which inhibits facilitated hexose transport and does not affect the hexokinase reaction (26-30), obliterated the saturable component of 2-deoxy-glucose uptake (Fig. 3). (*d*) Since the 3-min uptake of 2-deoxy-glucose determined in these studies is a measure of initial uptake rate, and since only a small fraction of the total sugar taken up at this time is in the form of free 2-deoxy-glucose (Table II), it is very unlikely that the capacity of the hexokinase reaction is exceeded. (*e*) Bernstein and Kipnis (10) have shown that adipocyte hexokinase is normal in rat adipocytes 4 h after the in vivo administration of dexamethasone, and we have found no effect of the glucocorticoid on hexokinase

activity after 3 h of in vitro incubation. (*f*) The data on glucose oxidation at maximal insulin levels (Fig. 1) indicate that the intracellular oxidative processes (including the hexokinase reaction) are comparable in treated and control cells. (*g*) Lastly, when 2-deoxy-glucose concentrations are increased (Fig. 6), dexamethasone-treated cells are capable of taking up much more 2-deoxy-glucose than during low sugar concentration (0.28 mM) and maximal insulin levels (5 ng/ml), as described in Fig. 5.

Since adipocytes isolated from dexamethasone-treated rats have a decreased ability to bind insulin (8), it seemed reasonable to believe that the dexamethasone-induced decrease in insulin-mediated glucose oxidation and transport was related to a similar dexamethasone-induced decrease in insulin binding. However, the data in Fig. 8 demonstrate that dexamethasone does not decrease insulin binding to adipocytes in vitro. These findings are in apparent contrast to the in vivo observations that isolated adipocytes and hepatocytes obtained from rats treated with dexamethasone have decreased insulin receptors (8). The explanation for this difference between the in vitro and in vivo effects of dexamethasone on insulin binding is not clear. Gavin et al. (42) have demonstrated in vitro that sustained high concentrations of insulin can lead to decreased numbers of insulin receptors on cultured lymphocytes and have suggested that this same phenomenon might operate in vivo. Consequently, one possibility is that in vivo dexamethasone induces increased plasma insulin levels, which lead to a decrease in insulin receptors on cells. Another possibility is that more time is needed for the effects of dexamethasone on insulin binding to develop than can be achieved in these in vitro studies. Obviously, other potential explanations exist.

The data clearly show that for any given amount of insulin bound, dexamethasone: treated adipocytes take up less 2-deoxy-glucose than untreated cells. However, since basal uptake rates are decreased to the same extent as are the insulin-mediated uptake rates (i.e., insulin-mediated increments in glucose transport are the same in both groups of cells) it is likely that the coupling between occupied insulin receptors and the glucose transport system is not affected by dexamethasone. However, the overall relationship between the amount of insulin bound and insulin action in both groups of cells is less clear. As seen in Fig. 9, maximal glucose transport occurs at an insulin concentration at which only 15% of the available receptors are occupied. This relationship between binding and function has been observed for other systems (43) and for other hormones (44, 45). Possible interpretations of this observation of apparent "spare" receptors have been recently reviewed (44, 45).

An additional interesting aspect of these studies was the observation that insulin had greater stimulatory effects on glucose oxidation than on transport. This suggested that insulin can promote oxidation by stimulating an intracellular step in the oxidative process as well as by increasing glucose transport. This idea was confirmed by finding that insulin could still promote glucose oxidation in the presence of cytochalasin B (which inhibits insulin's effects on transport). This posttransport effect adds a new dimension to the currently held concept that insulin promotes glucose oxidation by increasing transport (36, 37). Clearly, further studies will be necessary to delineate the locus and quantitative importance of this effect in terms of overall glucose metabolism.

In summary, these studies have demonstrated that dexamethasone in vitro inhibits glucose transport in both the basal and insulin-stimulated state, and appears not to affect primarily the intracellular enzymes responsible for glucose oxidation. Furthermore, in vitro, dexamethasone does not decrease insulin binding to adipocytes, in contrast to its in vivo effects. Thus, at least two mechanisms can be suggested to explain the insulin-resistant state associated with in vivo corticosteroid administration: (*a*) a decrease in insulin binding to cells, and (*b*) direct inhibition of the glucose transport system.

ACKNOWLEDGMENTS

The author wishes to thank Mrs. Helen Chang for her expert technical assistance.

This work was supported by grants from the National Institutes of Health, HL08506, and from the Veterans Administration.

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