Effect of Glucocorticoids on the Glucose Transport System of Isolated Fat Cells*

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Glucocorticoids inhibit glucose utilization by fat cells. The possibility that this effect results from altered glucose transport was investigated using an oil-centrifugation technique which allows a rapid (within 45 s) estimation of glucose or 3-O-methylglucose uptake by isolated fat cells. At high concentration (>25 μM), dexamethasone inhibited glucose uptake within 1 min of its addition to fat cells. Efflux of 3-O-methylglucose was also impaired by 0.1 mM dexamethasone. However, diminished glucose uptake was not a specific effect of glucocorticoids; high concentrations (0.1 mM) of 17β-estradiol, progesterone, and deoxycorticosterone produced a similar response in adipocytes.

At a more physiologic steroid concentration (0.1 μM), glucocorticoids inhibited glucose uptake in a time-dependent manner (maximum effect in 1 to 2 hours). This effect was specific for glucocorticoids since, under these conditions, glucose uptake was not changed by the non-glucocorticoid steroids. Lineweaver-Burk analysis showed that 0.1 μM dexamethasone treatment produced a decrease in V_max for glucose uptake but did not change the K_m. Hexokinase activity and ATP levels were not altered by this treatment, suggesting that processes involved in glucose phosphorylation were not affected. Dexamethasone treatment also caused a reduction in uptake of 3-O-methylglucose when assayed using a low sugar concentration (0.1 mM). At a high concentration (10 mM), uptake of the methyl sugar was only slightly less than normal in treated cells. Stimulation by insulin markedly enhanced uptake of glucose and 3-O-methylglucose by both treated and untreated cells. At a low hexose concentration (0.1 mM) and in the presence of insulin, sugar uptake by dexamethasone-treated cells was slightly less than control cells. Stimulation by insulin did however completely overcome the alteration in hexose uptake when larger concentrations of sugars (>5 mM) were used.

There was no detectable change in total protein synthesis during incubation of fat cells with dexamethasone. However, actinomycin C blocked the inhibitory effect of dexamethasone on glucose uptake. Cycloheximide, which caused a small inhibition in glucose uptake, prevented the full expression of the inhibitory effect of dexamethasone on glucose transport. These results indicate that dexamethasone alters the facilitated transport of glucose and, secondly, suggest that synthesis of RNA and protein is needed for glucocorticoid action.

Fat is one of the few tissues which will respond in vitro to glucocorticoids (2). A major effect resulting from glucocorticoid treatment of fat (3-9) and other tissues (10-12) is a reduction in glucose metabolism. It has been suggested that this response in fat is secondarily derived from a glucocorticoid-induced change in the glucose transport system (7, 9). However, evidence for this proposal is indirect since previous investigators studied the effectiveness of the transport system by measuring the products of glucose metabolism, a method which is dependent upon many steps besides glucose uptake. In addition, this approach does not provide information

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concerning the nature or specific characteristics of the alteration.

In the present work, the effect of dexamethasone and other glucocorticoids on the glucose transport system of isolated adipocytes was directly examined using an oil-centrifugation method (13) to rapidly measure hexose uptake (14). The alterations in glucose uptake induced by these steroids were studied as well as the mode of action underlying these changes.

EXPERIMENTAL PROCEDURE

Animals—Male Sprague-Dawley rats (Charles Rivers Co.) weighing 125 to 150 g were maintained on Purina Laboratory Chow and fed ad libitum. The animals were killed by a sharp blow to the head: the epididymal fat pads were removed and used for the preparation of isolated fat cells.
Isolation of Fat Cells—Intact, viable adipocytes were prepared by a slight modification of the collagenase method of Rodbell (15). Minced epididymal fat was incubated for 45 min at 37° in sugar-free Krebs-Ringer buffer (pH 7.4), modified by the use of 1.4 mM CaCl\(_2\) and the addition of 30 mg/ml of bovine albumin and 1 mg/ml of crude collagenase (type I). Following collagenase treatment, the isolated fat cells were washed three times and resuspended in the modified Krebs-Ringer buffer minus the collagenase. Fat cell numbers were estimated by determining the DNA content of a concentrated suspension of cells as previously described (16).

**Measurement of Glucose Uptake**—The fat cell suspension was further diluted with the albumin Krebs-Ringer buffer to give a final DNA concentration of 0.1 to 0.4 mg/ml. Under these conditions, the cell suspension was placed in the incubation medium (final dimethylsulfoxide concentration of 0.5%). Steroids were prepared for studies of time-dependent effects by dissolving in 1 ml of ethanol and subsequent dilution with 50 ml of 0.15 M NaCl. A 10-μl aliquot was added to the incubation medium (final ethanol concentration of 0.01%). Neither dimethylsulfoxide nor ethanol in the amounts used influenced glucose uptake.

Glucose uptake was rapidly determined using an oil-centrifugation method as previously described by Livingston and Lockwood (14). In brief, the assay was initiated by addition of the appropriate amount (25 μl to 100 μl) of [U-\(^{14}\)C]glucose (0.33 to 4.0 μCi/μmol) to the cell suspension from which a similar amount (25 μl to 130 μl) of cell-free infranatant was removed to maintain a constant volume of 0.5 ml. After addition of the sugar, the cell suspension and cell-free infranatant were centrifuged at 600 g for 1 min. The infranatant from 37° water bath. Twelve seconds before termination of the assay, the content of the assay tube was transferred to a 0.45-ml microcentrifuge tube that contained 75 μl of silicone oil (specific gravity of 0.97). The microcentrifuge tube was placed in a Beckman microturbine and the assay terminated by centrifugation at 10,000 × g for 15 s. In general, centrifugation was begun 47 s after the addition of the labeled sugar. Centrifugation almost immediately separates the cells from the incubation medium by forming a cell layer over the silicone oil which is interspersed between the cells and the aqueous incubation medium (13). The tubes were rapidly cut at the cell-oil interface and the radioactivity in the cell pellet determined as described (14). The oil-centrifugation method is available for the determination of cells from the incubation medium and apparently does not alter the cell's ability to synthesize lipids from glucose or respond to insulin (13).

The amount of glucose trapped extracellularly in the cell pellet was estimated by parallel experiments in which the proportion of insulin trapped by the pellet was compared with the total amount of insulin added to the assay. The ratio was determined by the addition of 10 μl of a 40.0 μg/ml solution of [methoxy-\(^{3}H\)julin (0.63 μCi/mg) to a 0.5 ml aliquot of cells following by isolation of the cells using the oil-centrifugation method. In the absence of insulin stimulation, the estimated amount of glucose trapped extracellularly was approximately 5% of the total radioactivity in the cell pellet following a 45-s incubation of cells with 5.0 mM glucose. The trapping was 32% of total pellet radioactivity when the cells were incubated under similar conditions with 0.1 mM glucose.

**Measurement of 3-O-Methylglucose Uptake**—Fat cells (1 to 2 μg of DNA) were incubated in the presence or absence of hormones as described for glucose uptake. However, in these experiments the oil-centrifugation method was modified as follows: Several regions of the e- centrifugation field were used to measure glucose uptake because of the rapid attainment of steady state conditions between extracellular and intracellular 3-O-methylglucose. Fat cells were cooled to 21° immediately before studies of sugar transport and maintained at this temperature during the assay for 3-O-methylglucose uptake. A 200-μl portion of the cell-free infranatant was removed from the floating layer of cells which held 100 μl of fat cell suspension. The assay was started by the addition of 25 μl of the appropriate concentration of 3-O-methyl-n-[\(^{3}H\)glucose (2.0 to 100 μCi/μmol) to the remaining cell suspension and gently shaken by hand. The assay was terminated by the addition of 200 μl of an ice-cold solution of 0.15 M NaCl which contained 5 mM phlorizin. The resulting 325 μl of cell suspension was rapidly transferred to a microcentrifuge tube containing silicone oil and the cells separated as described for the glucose uptake assay. A period of 8 s was required for separation of the cell pellet. After addition of 10 μl of insulin, trapping accounted for 25% of the total radioactivity. Addition of cold saline/phlorizin increased the sensitivity of the procedure by decreasing the concentration of sugar in the incubation medium which in turn reduced by one-third the amount of 3-O-methylglucose trapped in the cell pellet. The solution can be used in this manner since phlorizin impedes movement of sugar across the cell membrane (14, 17, 18).

**Efflux of 3-O-Methylglucose**—Fat cells (200 μg of DNA) in a plastic scintillation vial were incubated in a total volume of 6 ml with a final concentration of 0.5 mM 3-O-methyl-d-[\(^{3}H\)glucose (50 μCi/μmol) for 30 min at 37°. The cell suspension was then cooled to 21° and an 0.6-ml aliquot of cells was placed in a 2.0-ml centrifuge tube. Following centrifugation of the aliquot at 600 g for 1 min, the supernatant was carefully removed. The assay to measure efflux was started by the addition of 2.5 ml of phosphate Krebs-Ringer buffer, temperature 21°, which contained the indicated amount of steroid. The cell suspension was immediately mixed and shaken gently during the entire assay period. At various points of time, 300-μl aliquots of cell suspension were removed and the cells rapidly separated from the incubation buffer by the oil-centrifugation method. A comparison of the relative rates of efflux of 3-O-methylglucose between treatment and control groups was made by expressing the content of sugar remaining in the cell pellet following short periods of incubation (30 to 120 s) with that found at equilibrium (1 hour).

**Measurement of Distribution Spaces in Isolated Fat Cells**—The isolated fat cells (200 μg of DNA) were suspended at 3 ml of the phosphate Krebs-Ringer buffer either in the presence or absence of 0.1 μm dexamethasone. Following incubation at 37° for 2 hours under an atmosphere of 95% O\(_2\)/5% CO\(_2\) in a shaking water bath (Dubnoff), Glucose uptake was determined using an oil-centrifugation method as previously described by Livingston and Lockwood (14). In brief, the assay was initiated by addition of the appropriate amount (25 μl to 100 μl) of [U-\(^{14}\)C]glucose (0.33 to 4.0 μCi/μmol) to the cell suspension from which a similar amount (25 μl to 130 μl) of cell-free infranatant was removed to maintain a constant volume of 0.5 ml. After addition of the sugar, the cell suspension and cell-free infranatant were centrifuged at 600 g for 1 min. The infranatant removed and the cells resuspended and cooled to 4° by the addition of 1 ml of a buffer solution, pH 7.4, which contained 5 mM Tris, 0.1 mM EDTA, and 5 mM dithiothreitol. The cells were disrupted by a Dounce homogenizer and centrifuged at 1200 × g for 5 min at 4°. The fat cell was discarded while the supernatant and pellet were combined and assayed for hexokinase activity by determining the rate of phosphorylation of 2-deoxy-d-glucose. The assay consisted of incubating aliquots of the homogenate for 30 min at 37° in 50 mM Tris, pH 7.4, 17 mM MgCl\(_2\), 9 mM ATP, and 1.7 mM 2-deoxy-d-[\(^{14}\)C]glucose (1 μCi/μmol) for 5 min at 31°. The reaction was terminated by the addition of 4 ml of a solution containing 1 M glucose and 0.17 M ammonium hydroxide (18). The amount of phosphorylated 2-deoxyglucose was determined using Dounce 9-XA-100 as described by Gots and Bessman (19).

**Fat Cell ATP Content**—ATP content was determined in fat cells which had been previously incubated in the presence or absence of dexamethasone as described above for the hexokinase assay. Following the incubation, the fat cells were centrifuged at 600 × g for 1 min and the infranatant removed. Three milliliters of an ice-cold 5% perchloric acid solution were added and the cells were homogenized in a Dounce homogenizer. The fat cell was discarded after centrifugation at 1200 × g for 5 min at 4°. One milliliter of a 1 M Tris buffer, pH 8.0, was added to the perchloric acid extract and the pH of the solution adjusted to 7.5 in an ice bath by the slow addition of 10 N KOH. The precipitate formed during neutralization was removed by centrifugation and the ATP content of the supernatant was determined using a luciferase/luciferin assay as described by Weigel (20).

**Measurement of Distribution Spaces in Isolated Fat Cells**—The distribution spaces for \(^{3}H\)O, \(^{14}\)C-lactate, and [methoxy-\(^{3}H\)julin were determined by the procedure of Gliemann et al. (13). \(^{3}H\)O space was estimated by the addition of 25 μl of \(^{3}H\)O (0.63 μCi) to a 300-μl suspension of fat cells (1 μg of DNA) and incubated for 10 min at 37°. The cells were then isolated from the incubation medium by the oil-centrifugation method and the radioactivity in the cell pellet and
cell-free incubation medium were determined as described (14). The area space was estimated in a similar manner except that the cells were incubated for 30 min following addition of 10 μl of [3H]urea (60 μCi/1 μmol) before isolation of the cell pellet. The method for measuring the insulin uptake was described with the procedure used for measuring glucose uptake.

**Measurement of Protein Synthesis** Fat cells (60 μg of DNA) were incubated in 3-ml aliquots for 2 hours with L-[4,5-3H]leucine (1.4 μCi/μmol) and the balanced amino acid mixture described by Mine-}

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### RESULTS

**Rapid Effects of Glucocorticoids on Glucose Uptake**—Initial experiments were designed to determine whether glucocorticoids inhibit glucose metabolism in fat cells by acting directly on the transport system as reported in Novikoff hepatoma cells (28). The double reciprocal plot given in Fig. 1 shows that uptake of glucose is "immediately" decreased at dexamethasone concentrations of 0.025 mM or greater. Dixon and Webb analysis (29) demonstrated that the competition was noncompetitive and had an apparent Kᵢ of 0.1 mM (Fig. 1B).

Table I shows the results obtained when the efflux of 3-O-methylglucose from fat cells is measured in the presence of 0.1 mM dexamethasone or 5 mM phlorizin, a known inhibitor of glucose transport (14, 17, 18). Both phlorizin and dexamethasone decreased the rate of efflux as indicated by the larger amounts of 3-O-methylglucose present in treated cells during the first 2 min of the assay. These results suggest that high concentrations of dexamethasone rapidly and directly alter the hexose transport system of fat cells.

Glucocorticoids were not the only steroids which immediately reduced glucose uptake (Table II). At a concentration of 0.1 mM, several steroids including deoxycorticosterone, 17β-estradiol, and progesterone also markedly lowered uptake of glucose by fat cells. Apparently, high concentrations of many steroids can alter glucose transport and this effect probably results from a nonspecific association of these agents with the cell surface, an interaction analogous to that described in thymocytes (30).

**Time-dependent Glucocorticoid Effects on Glucose Transport**—Table II also shows the effects on glucose uptake when adipocytes were preincubated for 2 hours in the presence of various steroids at a much lower concentration (0.1 μM). Under these conditions, only dexamethasone and hydrocortisone significantly altered glucose uptake. The other steroids, which at high concentrations produced an immediate effect on glucose uptake, did not alter this system when used under these conditions. The lack of response to cortisone has been previously reported in other target tissues (31, 32) and probably results from an inability to convert cortisone to its more potent form.
The fat cells were treated with the steroids under two sets of conditions. First, addition of 0.1 mM concentration of steroid to the cells was immediately followed by measurement of glucose uptake. Secondly, to determine the effects of 0.1 μM steroids, the cells were preincubated for 2 hours with this concentration of the various steroids before glucose uptake was determined. Glucose uptake was measured in both conditions at 37°C using 0.5 mM glucose and an incubation time period of 45 s (see "Experimental Procedure"). Values are given as percentage of control (100%) and represent mean ± S.E. of three experiments.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Glucose uptake (μmol/mg)</th>
<th>Preincubation with 0.1 mM steroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone</td>
<td>54 ± 7</td>
<td>65 ± 7</td>
</tr>
<tr>
<td>Cortisol</td>
<td>35 ± 6</td>
<td>81 ± 3</td>
</tr>
<tr>
<td>Cortisone</td>
<td>92 ± 5</td>
<td></td>
</tr>
<tr>
<td>Deoxycorticosterone</td>
<td>46 ± 6</td>
<td>102 ± 2</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>46 ± 9</td>
<td>102 ± 6</td>
</tr>
<tr>
<td>Progesterone</td>
<td>55 ± 2</td>
<td>104 ± 8</td>
</tr>
<tr>
<td>Testosterone</td>
<td>97 ± 6</td>
<td>113 ± 8</td>
</tr>
</tbody>
</table>

*a Necessary amount of cortisone required to produce a 0.1 mM concentration when added to the fat cell suspension would not remain in solution.

The action of dexamethasone at a concentration of 0.1 μM was dependent upon the time of incubation with cells (Fig. 2). No "immediate" alteration in glucose uptake occurred; instead, the first consistent effect was found after a 30-min treatment of the cells with the hormone. Maximum inhibition was obtained between 1 and 2 hours of incubation. Therefore, a period of 2 hours was routinely used to study the possible effects of small concentrations of dexamethasone on fat cells. The inhibition produced by dexamethasone under these conditions was further characterized by double reciprocal analysis shown in Fig. 3. The alteration apparently involves only a decrease in the maximum velocity (V_max) for uptake since the K_m did not change.¹

Bernstein (33) recently showed that dexamethasone reduces the level of hexokinase in rat adipose tissue after 18 hours of treatment. However, in the present study, dexamethasone treatment for 2 hours did not alter hexokinase activity. Homogenates prepared from fat cells incubated in the presence or absence of 0.1 μM dexamethasone had hexokinase activities, respectively, of 8.67 ± 0.8 and 8.07 ± 0.75 nmol of 2-deoxyglucose phosphorylated per 5 min per μg of DNA (mean ± S.E. of three separate experiments). Also, the intracellular ATP levels are not changed by dexamethasone under the conditions used to show the alteration in glucose uptake. The levels of ATP expressed as nanomoles per μg of DNA in control and dexamethasone-treated cells were respectively 0.49 ± 0.05 and 0.52 ± 0.03 (mean ± S.E. of three experiments).

Insulin stimulation of glucose uptake and its effect on dexamethasone inhibition is shown in Fig. 4. At a very low glucose concentration (0.1 mM), insulin caused a marked increase in the rate of uptake for both treated and untreated cells. Insulin stimulation almost completely overcame the steroid inhibition when uptake was assayed at 0.1 μM glucose (Fig. 4A). When glucose uptake was measured by incubation of steroid-treated and untreated cells with 5.0 μM glucose, insulin stimulation caused a similar amount of uptake for both cell types (Fig. 4B).

The results provided by the studies of glucose uptake suggest that glucocorticoids alter the glucose transport system since the assay for uptake is based on very short incubation periods. Confirmation of this suggestion was obtained by measuring the uptake of 3-O-methylglucose (Fig. 5). When the uptake assay was carried out with 0.1 mM 3-O-methylglucose in the absence of insulin, the cells treated with 0.1 μM dexamethasone for 2 hours did not transport as much of the hexose as untreated cells (Fig. 5A). Insulin stimulation reversed the majority of this inhibition. Uptake of 3-O-methylglucose assayed with a 100-

¹K_m is defined as the concentration of glucose which gives one-half V_max.
FIG. 4. Stimulation of glucose uptake by insulin in dexamethasone-treated and untreated cells. Fat cells were preincubated for 2 hours at 37° in the presence (●) or absence (○) of 0.1 μM dexamethasone. During the final 30 min of the preincubation, 1000 microunits/ml of insulin were added (solid lines). Glucose uptake was determined following the preincubation by incubation of cells with 0.1 mM glucose (A) and 5 mM glucose (B) for the length of time indicated (see "Experimental Procedure"). Results are the mean of six determinations. † indicates a p < 0.05 by paired t test.

Fig. 5. Effect of dexamethasone and insulin on 3-O-methylglucose uptake in fat cells. The cells were treated with 0.1 μM dexamethasone and 1000 microunits/ml of insulin as described in legend to Fig. 4. At the end of the preincubation, 3-O-methylglucose uptake was determined (see "Experimental Procedure") using two concentrations of sugar in the assay: A, 0.1 mM and B, 10.0 mM. The results are mean of six separate determinations. † represents a statistical significance of p < 0.05 by paired t analysis.

absence of any detectable insulin resistance. Other studies have suggested that glucocorticoids produce an insulin-resistant state and have shown evidence that 125I-labeled insulin binding by adipose tissue is reduced (34). The results in this study indicate that, if insulin resistance is caused by these steroids, a longer period of time is required than that needed to influence hexose transport.

The studies above demonstrate a decrease in hexose transport following glucocorticoid treatment but do not provide information of the mechanism which mediates this change. Since it has been proposed that RNA formation and protein synthesis are early steps in glucocorticoid action (35-37), the effects of inhibitors of these processes on the response of fat cells to dexamethasone was studied (Table V). As shown, incubation of the cells for 2 hours with cycloheximide decreased glucose uptake although the inhibition was not as great as that found with dexamethasone. Addition of dexamethasone together with cycloheximide did not enhance the effects of
Conducted using a concentration of 7 x 10^{-10} M "26-1-labeled insulin (experiments). Insulin binding in these experiments was con-
metabolize glucose was diminished by glucocorticoid treat-
appear to be altered by dexamethasone under the conditions
respectively 1,383 + 80 cpm and 1,473 + 148 cpm of "H-labeled protein present in the plasma membrane of the fat
cells was determined (see "Experimental Procedure"). In addition, the amount of 
protein in the presence of 0.1 MM dexamethasone was provided (see "Experimental Procedure"). During a 2-hour incubation, steroid-treated cells incorporated 17,548 + 504 cpm into total protein (see "Experimental Procedure"). Cycloheximide, 0.1 mM + dexamethasone, 0.1 FM 

Effects of inhibitors of protein synthesis on glucose uptake and
dexamethasone action

Fat cells were incubated for 2 hours at 37° with the indicated agents. At the end of the incubation period, glucose uptake was determined as described in Table II. Values are given as percentage of control (100%) and represent mean ± S.E. of three experiments.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Glucose uptake (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (Control)</td>
<td>100</td>
</tr>
<tr>
<td>Dexamethasone, 0.1 μM</td>
<td>58 ± 9</td>
</tr>
<tr>
<td>Cycloheximide, 0.1 mM</td>
<td>77 ± 9</td>
</tr>
<tr>
<td>Cycloheximide, 0.1 mM + dexamethasone, 0.1 μM</td>
<td>78 ± 6</td>
</tr>
<tr>
<td>Actinomycin C, 0.2 μg/ml</td>
<td>114 ± 19</td>
</tr>
<tr>
<td>Actinomycin C, 0.2 μg/ml + dexamethasone, 0.1 μM</td>
<td>107 ± 14</td>
</tr>
</tbody>
</table>

Dexamethasone altered glucose uptake in the adipocytes under two distinct acts of conditions. Glucose uptake by untreated cells was "immediately" decreased upon the addition of high concentrations of dexamethasone. This effect apparently stems from an alteration in the glucose transport system since the rate of efflux of 3-O-methylglucose from control cells was also diminished when a large amount of the steroid was present. These findings confirm in part to those reported by Plageman and Renner (28) who showed direct competition between glucose and large concentrations of prednisolone for glucose uptake by Novikoff hepatoma cells. The inhibition in fat cells differed from that described in hepatoma cells by its noncompetitive characteristics and lower K_i. Although Plageman and Renner (28) suggested a physiologic role for this direct effect, the high concentrations of glucocorticoids required in fat cells coupled with the ability of non-glucocorticoid steroids to produce the same response strongly argues against the possibility that inhibition of hexose uptake is normally mediated in this manner. Instead, it is probable that this action of high concentrations of both glucocorticoid and non-glucocorticoid steroids results from their well known abilities to interact with lipids that are present in the plasma membrane of cells (39).

The specific action of glucocorticoids on glucose transport was found when the cells were preincubated with a low concentration (0.1 μM) of these hormones for 2 hours. The non-glucocorticoid steroids did not produce any effects on this system when preincubated in low concentrations with fat cells. Specificity for active glucocorticoids and the requirement of time for development of the inhibition are in agreement with earlier studies in adipose tissue that documented an effect of these steroids on glucose metabolism (3-9).

Lineweaver-Burk analysis indicated that dexamethasone treatment for 2 hours caused a decrease in V_max for glucose uptake but did not alter the K_m. This interpretation must be viewed with caution since the analysis was carried out using a narrow glucose concentration range. However, when glucose concentrations of less than 5 mM are used, it does appear that dexamethasone treatment diminishes the capacity of fat cells, under basal conditions, to take up glucose.

The processes involved in phosphorylation of glucose do not appear to be altered by dexamethasone under the conditions

cycloheximide and resulted in less inhibition than that produced by dexamethasone alone. In contrast to the findings with cycloheximide, actinomycin C did not inhibit glucose uptake. However, this antibiotic blocked the inhibitory effect of dexamethasone on glucose transport.

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used to treat the cells in the present study. Both hexokinase activity and the level of ATP in dexamethasone-treated cells were similar to those found in control adipocytes. Although dexamethasone lowers the activities of both hexokinase isoenzymes present in fat, this effect reportedly requires incubation periods of over 4 hours (33). Similarly, Yarke (9) did not find a change in ATP levels of fat pads incubated with dexamethasone although results of this study are difficult to interpret because of the other cell types present in the tissue preparation used.

Direct evidence which indicates an alteration in glucose transport was obtained by comparing the abilities of treated and untreated cells to take up 3-O-methylglucose, a sugar which cannot be phosphorylated by adipose tissue (14, 17). Incubation of fat cells for 2 hours with 0.1 mM dexamethasone decreased the basal uptake of 3-O-methylglucose when the assay was conducted with a low concentration of the sugar (0.1 mM). These conditions were shown by Czech and Fain (7) to be ideal for demonstrating the effect of dexamethasone on glucose metabolism in fat. This effect did not appear to involve a change in the water space of the fat cell nor was the extracellular space in the isolated cell pellet altered by dexamethasone. At a much higher concentration of 3-O-methylglucose (10 mM), the uptake by treated cells tended to be less than control cells but the difference was not statistically significant. Stimulation of the cells by insulin, either partially or completely, overcame the alterations induced by dexamethasone. When uptake was assayed using 0.1 mM 3-O-methylglucose, insulin partially corrected the diminished transport; however, insulin-stimulated uptake, when assayed at a sugar concentration of 10 mM, was exactly the same in treated and untreated cells.

Insulin overcame the alteration in glucose uptake in much the same fashion as that found for uptake of 3-O-methylglucose. It has been shown that high glucose concentrations in conjunction with insulin obscures the defect in glucose metabolism induced by glucocorticoids (5, 7). Czech and Fain (7) suggested that this occurs because the rate of glucose influx induced under these conditions changes the rate-limiting step from glucose transport to processes responsible for the formation of glucose metabolites. However, our findings that insulin stimulation can overcome the alterations in both glucose and 3-O-methylglucose uptake argues against this possibility. Instead, these results suggest that very high rates of sugar transport obscure the relatively small decrease in uptake caused by dexamethasone treatment. Insulin does not appear to prevent the uptake by treated cells to take up 3-O-methylglucose, a sugar which cannot be phosphorylated by adipose tissue (14, 17). This report showed that elevated plasma levels of corticosterone in the rat are associated both with insulin resistance and a change in binding of 125I-labeled insulin by liver membranes (34). However, Bennett and Cuatrecasas (41) did not find an alteration in insulin binding to adipocytes isolated from rats given prednisolone. The findings of the present study show that, if these steroids alter the biologic response of fat cells to insulin, a period of incubation longer than 2 hours is required.

The mechanism for the action of dexamethasone was investigated by the use of inhibitors of protein synthesis. Although cycloheximide caused a small reduction in glucose uptake, it prevented the full expression of the inhibitory effect of the glucocorticoid. In addition, actinomycin, which did not inhibit glucose uptake, blocked the action of dexamethasone. These findings agree in part with those from previous studies of glucose metabolism in fat. For example, actinomycin prevented dexamethasone from decreasing CO2 production from glucose (42). These studies also demonstrated that cycloheximide (6, 42) inhibited glucose metabolism and that its effect was not additive to that of dexamethasone (42).

Because of the similarities between the effects of cycloheximide and dexamethasone on glucose oxidation, it has been suggested that the steroid acts by inhibiting the synthesis of a protein important in glucose metabolism (7). That glucocorticoids can inhibit protein synthesis in the liver has been recently demonstrated by Kim and Kim (43). However, our measurements of total protein synthesis by adipocytes indicate a similar amount of synthesis by dexamethasone-treated and untreated cells. In addition, dexamethasone did not produce a detectable alteration in the amount of newly synthesized protein associated with the adipocyte plasma membrane.

These findings do not disprove the proposal that dexamethasone decreases the synthesis of a specific protein but it is important to note that 0.1 mM cycloheximide, which caused a 92% inhibition in total protein synthesis in our studies, failed to decrease glucose transport to the same degree as dexamethasone. Also, the ability of cycloheximide to reduce the dexamethasone response of the fat cell suggests that it may block mediation of the glucocorticoid effect. Since cycloheximide alone produces a small decrease in glucose uptake, it is difficult to judge the extent of the block. However, this finding argues against the concept that dexamethasone acts by inhibiting the synthesis of a protein(s) required for hexose transport.

Results of the present study and those previously reported by other workers (41) indicate that RNA synthesis is needed for attainment of the glucocorticoid effect. Furthermore, our findings in experiments with cycloheximide suggest that protein synthesis is required, i.e. that dexamethasone promotes the synthesis of a protein inhibitor to glucose transport. This proposed mechanism is similar to that recently suggested for the action of cortisol on glucose metabolism in thymocytes (35-37). Extensive studies in these cells show that cycloheximide and actinomycin block the inhibitory effect of glucocorticoids on glucose transport. It is also of interest that cycloheximide did not alter glucose transport but markedly inhibited glucose metabolism (37). A similar situation may exist in the fat cell to explain the moderate to marked inhibition of glucose metabolism by cycloheximide while producing only a small inhibition of glucose transport. This would suggest that the alterations in glucose metabolism induced by dexamethasone and cycloheximide involve different sites or mechanisms of action. It is apparent that the mechanism which underlies the action of glucocorticoids requires further study but the conclusive demonstration that dexamethasone alters the hexose transport system constitutes a necessary step in understanding glucocorticoid effects in adipose tissue.

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