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Dexamethasone inhibition of cholesterol-stimulated glucose transport in 3T3-L1 cells

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This study investigates the effects of dexamethasone on cholesterol-stimulated glucose uptake in confluent 3T3-L1 fibroblasts. Dexamethasone pretreatment blocked both insulin-stimulated glucose transport, and the increase produced by exogenous free cholesterol. The dexamethasone effect was inhibited in both cases by cycloheximide. Concentrations of cholesterol which increased glucose transport failed to produce a significant increase in measurable free cholesterol content in the plasma membrane.

Introduction

The mechanism by which dexamethasone inhibits glucose transport has been variously attributed to decreases in insulin receptor number [1,2], decreased movement of receptors and/or transporters from within the cell into the plasma membrane [3] and to dexamethasone induced changes in the lipids of the plasma membrane [4]. An effect of corticosteroids on membrane lipids has been reported from this laboratory in studies which have demonstrated a dexamethasone induced increase in the sphingomyelin content of rat fat cell ghosts [5], human leukocytes [6], and a plasma membrane enriched fraction of 3T3-L1 fibroblasts [7]. In addition to the effects on sphingomyelin, the cholesterol content of fat cell ghosts obtained from rat epididymal fat pads was decreased following adrenalectomy and increased by in vitro exposure to dexamethasone [8]. Changes in sphingomyelin and cholesterol have also been reported in HeLa cells exposed to corticosteroids in vitro [9].

An increase in choline containing lipids, and particularly sphingomyelin, was reported by Dawson et al. [10] to be inhibitory to diacylglycerol-stimulated phospholipases. The effect of altered lipids on transport has been suggested to result from changes in membrane fluidity perhaps in isolated domains of the membrane [11]. Cholesterol alterations have been demonstrated to influence glucose transport [11,12]. The present study was undertaken to determine the effect of incubations with cholesterol on glucose uptake by 3T3-L1 fibroblasts, and to determine whether dexamethasone would have an effect on glucose transport increased by incubation of the cells with added cholesterol.

Materials and Methods

Defined fetal bovine calf serum was obtained from HyClone Laboratories and Dulbecco's Modified Eagle's Medium was purchased from Grand Island Biological Company. Disposable plastic tissue culture supplies were obtained from Falcon

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Plastics. Insulin, dexamethasone, free cholesterol (99% pure), trichloroacetic acid, and non-radioactive 2-deoxyglucose were purchased from Sigma. Radioactive 2-deoxy[^{14}C]glucose was obtained from New England Nuclear Research Products. Reagent grade or better solvents were purchased from Mallinckrodt and were redistilled in glass prior to use. Scint-A from Packard was used as a liquid scintillation cocktail.

3T3-L1 fibroblasts were obtained from Harold Green (MIT). Cells were maintained in Dulbecco's Modified Eagle's Medium with 10% defined fetal calf serum and were grown at 37°C in an atmosphere of 5% CO_2 on 100 × 10 mm plastic tissue culture plates.

Media were changed three times weekly. The cells were subcultured before reaching confluency and experiments were carried out two or three days after confluence prior to conversion to adipocytes. The culture media was changed at least 24 h prior to starting an experiment.

Glucose uptake was determined by measuring 2-deoxy[^{14}C]glucose incorporation by 3T3-L1 fibroblasts [13]. This procedure was carried out in four experimental groups: Control, dexamethasone (10^{-7} M) treated, insulin ($4.2 \cdot 10^{-8} - 4.2 \cdot 10^{-5}$ M) treated, and dexamethasone plus insulin treated. Cells were incubated with dexamethasone for 24 h. 60 min prior to termination of the experiment, the cells were washed and the media replaced with Krebs-Ringer phosphate buffer with dexamethasone added again to appropriate groups (128 mM NaCl, 1.4 mM $MgSO_4$, 5.2 mM KCl, 10 mM Na_2HPO_4, and 1.4 mM $CaCl_2$; pH 7.4). Insulin was added to appropriate groups for the final 30 min of each experiment. During the last 10 min of this incubation 0.2 mM 2-deoxy[^{14}C] glucose (0.1 μCi/μmol) was added. The incubation was stopped by pouring off the buffer and washing with cold Krebs Ringer phosphate buffer four times. Trichloroacetic acid (5%) was added and the cells were allowed to stand for 30–60 minutes at 4°C. The trichloroacetic acid was collected and pooled with an additional 3 ml trichloroacetic acid wash. The total trichloroacetic acid was aliquoted in duplicate and the radioactivity determined. Protein determination was according to Lowry et al. [14].

Free cholesterol (1 mg dry weight/ml) was added to Dulbecco's Modified Eagle's Media that contained 10% fetal bovine calf serum. Controls and the above mixture were placed in a 37°C water bath and shaken mechanically overnight. The free cholesterol enriched media and controls were filtered through a Nalgene type S sterilization filter unit with a 0.45 μm filter. The cholesterol-enriched sterile media were added to the cells, the mixture was then incubated for 4 h. At the end of the incubation the cholesterol-enriched media were removed and the cells were washed with Krebs-Ringer phosphate buffer prior to the glucose transport studies. The cholesterol content of the incubation media was determined by gas-liquid chromatography by forming TMS derivatives and injecting them onto a column containing 3% OV-1 coated on 100/120 mesh Gas Chrom Q [8]. Plasma membrane enriched fractions were prepared using the discontinuous ficoll gradient system reported by Whittenberger and Glaser [15].

Results

The effect of insulin upon 2-deoxyglucose uptake is seen in Fig. 1. Although an increase in glucose uptake was induced by as little as 65 μU/ml ($4.2 \cdot 10^{-8}$ M) insulin, a significant increase was seen with 6.5 mU/ml ($4.2 \cdot 10^{-6}$ M) and at 65 mU/ml ($4.2 \cdot 10^{-5}$ M). Although the latter is an unphysiologic concentration, it produced the greatest increase in transport. This high concentration may have effects on receptors not specific for insulin, but as the study was directed toward inhibition of transport and not specifically the effects of insulin, the excess was employed.

The effect of the free cholesterol concentration of the media (in which the fibroblasts were incubated) on glucose transport is seen in Fig. 2. The level of free cholesterol in the control media containing 10% fetal bovine calf serum was determined to be $3.1 \cdot 10^{-5}$ M (12.0 μg/ml). Although lower concentrations had no significant effect, an increase in the concentration of free cholesterol to $9.3 \cdot 10^{-5}$ M (36 μg/ml) resulted in a very significant increase in glucose transport ($P < 0.001$).

Inhibition of the cholesterol-stimulated glucose transport by prior incubation with dexamethasone

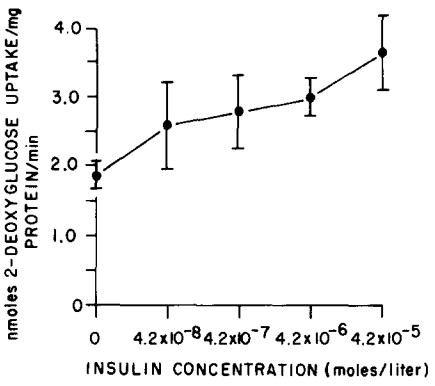

Fig. 1. The effect of increasing concentrations of insulin on glucose uptake in confluent 3T3-L1 cells. Cells were incubated with insulin for 30 min. 2-Deoxy[^{14}C]glucose was added the last 10 min of the incubation to determine glucose uptake. To assure a near maximum insulin response, subsequent experiments were undertaken using $4.2 \cdot 10^{-5}$ M insulin ($n = 5$, mean ± S.E.).

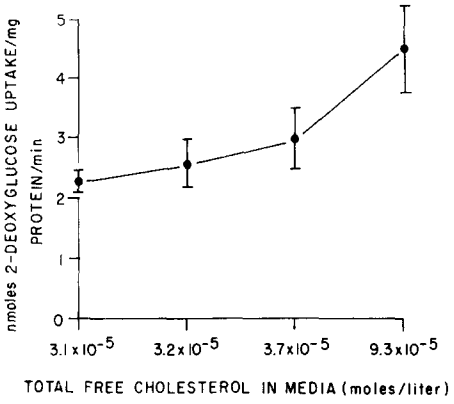

Fig. 2. Effect of increasing concentrations of free cholesterol in the incubation media on glucose transport in 3T3-L1 fibroblasts. Incubation with cholesterol was 4 h prior to determination of glucose uptake (mean ± S.E.; $P < 0.001$ for $9.3 \cdot 10^{-5}$ M concentration compared to controls, $n = 27$; other concentrations $n = 6$). Control level of cholesterol was $3.1 \cdot 10^{-5}$ M which was the amount of free cholesterol isolated from the tissue culture media. For description of preparation of cholesterol-enriched media see Methods.

is shown in Fig. 3. There was no effect of 10^{-10} M or 10^{-9} M dexamethasone upon 2-deoxyglucose uptake by the fibroblasts incubated with $9.3 \cdot 10^{-5}$ M free cholesterol, but 10^{-8} M dexamethasone produced a significant decrease in uptake and there was a further effect when the concentration of dexamethasone was increased to 10^{-7} M.

Table I summarizes the results of experiments in which glucose uptake was stimulated by insulin or a 4 h incubation with cholesterol. Prior incubation with dexamethasone inhibited stimulation by either insulin, added cholesterol, or control levels of glucose transport. The dexamethasone effect was abolished when the fibroblasts were previously incubated with cycloheximide.

TABLE I

CYCLOHEXIMIDE INHIBITION OF DEXAMETHASONE EFFECT ON GLUCOSE TRANSPORT

3T3-L1 fibroblasts were preincubated 24 h with and without 10^{-7} M dexamethasone (Dex). Cycloheximide (Cyclo), 2 μg/ml ($7.1 \cdot 10^{-6}$ M) was added to appropriate groups 24 h prior to determination of glucose uptake. Appropriate groups were incubated for 4 h with media containing increased free cholesterol (Chol) (see Methods) and/or $4.2 \cdot 10^{-5}$ M insulin for 30 min. 2-Deoxy[^{14}C]glucose was added the last 10 min of the incubation to determine glucose uptake.

Treatment	2-Deoxyglucose uptake (nmol/mg protein per min) (mean ± S.E.)	n	P value (control vs. activator)	P value (activator vs. Dex treatment)
Control	1.94 ± 0.05	12		
Dexamethasone	0.90 ± 0.17	12		< 0.001
Insulin	4.10 ± 0.38	12	< 0.001	
Insulin + Dex	2.13 ± 0.12	12		< 0.001
Cholesterol	2.98 ± 0.24	12	< 0.001	
Cholesterol + Dex	1.06 ± 0.06	12		< 0.001
Chol + Dex + Cyclo	2.67 ± 0.19	12		> 0.2
Cycloheximide	1.83 ± 0.07	6	> 0.5	

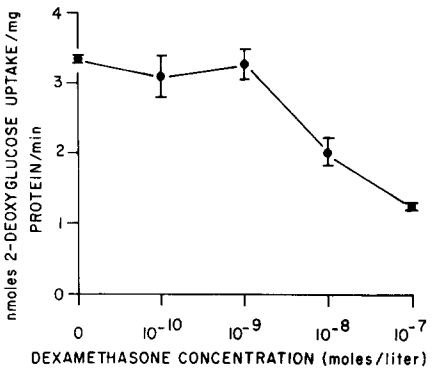

Fig. 3. Effect of increasing concentrations of dexamethasone on cholesterol-stimulated glucose transport. 3T3-L1 fibroblasts were incubated with various concentrations of dexamethasone for 24 h. All groups of cells were incubated with media containing $9.3 \cdot 10^{-5}$ M free cholesterol for 4 h after exposure to dexamethasone for 24 h ($n = 2$–4; mean ± S.E.).

Studies carried out to determine whether an increase in membrane free cholesterol was responsible for the effect on glucose uptake were inconclusive. Measurement of plasma membrane free cholesterol by gas chromatography demonstrated an insignificant increase in membrane cholesterol after incubation with a concentration of exogenous cholesterol which stimulated glucose transport (74.6 ± 5.4 vs. 80.7 ± 7.1 μg free cholesterol per mg protein after the 4 h incubation).

As dexamethasone may influence protein synthesis, whole cell protein content used to standardize glucose uptake was compared between experimental groups with the following results: control, 1.73 ± 0.08 mg/plate; insulin, 1.75 ± 0.08; dexamethasone, 1.79 ± 0.08; dexamethasone and insulin combined, 1.75 ± 0.07 (mean ± S.E., $n = 10$). There was, therefore, no significant effect of hormonal treatment upon total protein content of the cells under the experimental conditions employed.

Discussion

Neither the mechanism by which dexamethasone inhibits glucose uptake nor that mechanism by which addition of cholesterol to cells in vitro increases glucose transport is presently known. At the time that this study was initiated, it was hypothesized that as dexamethasone inhibits insulin-stimulated uptake, inhibition of cholesterol-stimulated uptake might suggest that both agents share some part of a common pathway. The results obtained are consistent with this hypothesis. As was found by other workers using erythrocytes [12] and 3T3 cells [11], an increase in total free cholesterol in the media in which the 3T3-L1 fibroblasts were incubated produced a significant increase in 2-deoxyglucose uptake. Increased uptake was inhibited by a preliminary incubation of the cells with concentrations of dexamethasone which also inhibited insulin-stimulated uptake. Addition of cycloheximide to the incubation media with the dexamethasone abolished the effect of the steroid on cholesterol-stimulated uptake. Therefore, new protein synthesis was necessary, as has previously been shown for the steroid effect on glucose uptake [16]. Recent studies have suggested that the corticosteroid effect may be mediated by sphingolipid modification of protein kinase C activity [17], but no investigation of this possibility is included in this study. Although 2-deoxy uptake cannot be directly correlated with glucose transport, studies by Putton et al. [18] using the same cell type employed in these studies have shown good correlation between 2-deoxyglucose uptake and 3-*O*-methylglucose transport.

The mechanism by which incubations with cholesterol increase glucose uptake by the cells is still not clear. Although there was no significant increase in free cholesterol in the plasma membrane enriched fraction studied in this investigation, the findings do not rule out the possibility that small changes or localization of the cholesterol in the membrane may be responsible for the observed increase in glucose uptake. It is clear, however, that dexamethasone inhibits cholesterol- and insulin-stimulated glucose uptake. Further studies will be necessary to more clearly determine the mechanisms involved.

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