GLUCOCORTICOIDS: COMPETITIVE INHIBITION OF GLUCOSE TRANSPORT

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Received November 29, 1971

SUMMARY

Prednisolone competitively inhibits the transport of 2-deoxy-D-glucose by cultured Novikoff rat hepatoma cells without affecting deoxyglucose phosphorylation. The Ki for the inhibition (2 mM) is about the same as the Km for deoxyglucose transport. Transport is maximally inhibited immediately upon addition of the drug. Glucose metabolism by the cells is inhibited in direct proportion to the inhibition of glucose uptake.

Glucocorticoids markedly inhibit the metabolism of glucose when administered to animals or to tissue cell suspensions or cell cultures (1-6). It has been postulated that the effect is due to a direct or indirect inhibition of glucose uptake, since dexamethasone has no effect on the level of hexokinase in glucocorticoid-sensitive cultured lymphosarcoma cells or on the activity of the enzyme (6). The inhibition of glucose uptake may be the primary action of glucocorticoids and other effects on macromolecular synthesis may be related to a decreased availability of nucleoside triphosphates as a consequence of the decreased metabolism of glucose (5-8). However, although the inhibition of glucose uptake by lymphosarcoma cells or rat thymus cells is more rapid than the effect on nucleic acid or protein synthesis, it seems to require a definite time period of incubation to develop (4, 5). In contrast, the present results show

that glucose metabolism by Novikoff rat hepatoma cells growing in suspension culture is maximally inhibited immediately upon addition of prednisolone. Kinetic studies indicate that this effect is due to a competitive inhibition of glucose transport by prednisolone.

MATERIALS AND METHODS

Novikoff rat hepatoma cells (subline N1S1-67) were propagated in suspension culture (9) and cells in the exponential phase were harvested by centrifugation and suspended to 2×10^6 cells/ml in glucose-free basal medium 42 (BM42; 10) or glucose-free BM42 (BM42-HEPES) in which the bicarbonate had been replaced with 50 mM N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES, Calbiochem). Prednisolone-21-sodium succinate (Sigma) and ^{14}C-UL-D-glucose and ^{14}C-UL-2-deoxy-D-glucose (International Chemical and Nuclear Corp.) were added as indicated in the appropriate experiments and the suspensions incubated at 37° on a gyrotory shaker. Duplicate samples of suspension were analyzed as follows (11): (a) for radioactivity in total cell material: The cells were collected by centrifugation, washed once in 5 ml BSS (11) and then suspended in 0.2 ml 0.5 N trichloroacetic acid. (b) for radioactivity in acid-insoluble material: The cells were immediately frozen in a bath of solid CO_2 in ethanol. Later the samples were thawed, mixed with perchloric acid at 0° and the precipitate was washed repeatedly with 0.5 N trichloroacetic acid (9) and finally suspended in 0.1 ml of the same. The samples of (a) and (b) were heated at 70° for 30 min and then analyzed for radioactivity (10). Cell-free extracts were prepared and assayed for hexokinase activity as described elsewhere (12) or for tyrosine amino-transferase activity as described by Hayashi et al. (13).

RESULTS AND DISCUSSION

The results in Fig. 1 demonstrate that the incorporation of ^{14}C-glucose into total cell material (acid-soluble plus acid-insoluble) and into acid-insoluble material as well as its conversion to extracellular lactate and CO_2 were inhibited within 5 min after the addition of prednisolone to the cells. The inhibitions were proportional to the prednisolone concentration added and all processes were affected to about the same extent by the various concentrations. Further, the distribution of label among the components of the acid-soluble pool and macromolecules was not affected by prednisolone (not shown). As documented elsewhere (12) 50-60% of the label in the acid-soluble pool derived from ^{14}C-glucose is located in nucleotides and 50-60% of the label in acid-insoluble material is associated with nucleic acids regardless of the glucose concentration in the medium. Between 8 and 15% of the label in acid-insoluble material is associated with lipids and the remainder with proteins and glycoproteins. The finding that the intracellular fate of the glucose taken up by the cells was not affected by prednisolone coupled with the observation that transport is the rate-limiting step in the phosphorylation and metabolism of glucose by N1S1-67 (12) suggests that the hormone affects glucose metabolism by N1S1-67 cells by inhibiting its transport into the cells. This conclusion is further supported by the finding that prednisolone had no effect on the phosphorylation of glucose by cell-free extracts from N1S1-67 cells even at a concentration of 20 mM (not shown). It is further supported by results from studies on the effect of prednisolone on deoxyglucose transport (Fig. 2). Deoxyglucose was not metabolized by N1S1-67 cells beyond deoxyglucose-6-phosphate and 6-phospho-deoxygluconate (Fig. 2B) and was not incorporated into acid-insoluble material. However, free deoxyglucose also accumulated intracellularly (Fig. 2B), probably because deoxyglucose is phosphorylated by the hexokinase from

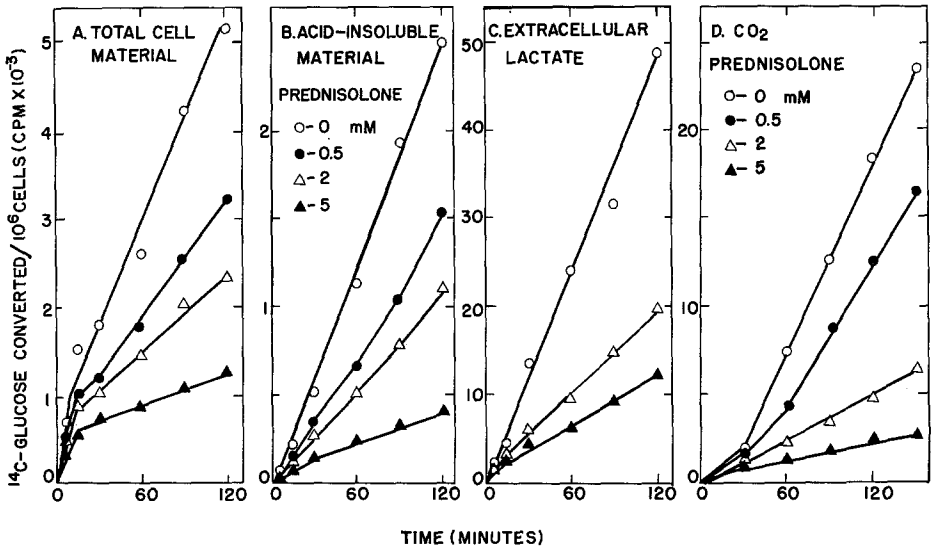

Fig. 1. Effect of prednisolone on glucose metabolism by N1S1-67 cells. (A-C) Samples of a suspension of 2 x 10^6 cells/ml of glucose-free BM42 were supplemented with the indicated concentrations of prednisolone and within 1 min thereafter (0 time) with 2 mM ^{14}C-UL-glucose (225 cpm/nmole). Duplicate 0.5-ml samples were analyzed for radioactivity in total cell material (A) or in acid-insoluble material (B). All points represent averages of the duplicate samples. Production of labeled lactate (C) was estimated as follows (12): 25-µl samples of culture fluid were analyzed by ascending chromatography on 3 MM Whatman paper with a solvent composed of ethylacetate, glacial acetic acid and H_2O (3:1:1; v/v) and the lactate and glucose regions were analyzed for radioactivity. (D) Samples of 10 ml of a suspension of 2 x 10^6 cells/ml of glucose-free BM42-HEPES were supplemented with the indicated concentrations of prednisolone and 50 µM ^{14}C-UL-glucose (9 cpm/pmole) and incubated in $^{14}CO_2$ collector flasks (Wheaton Glass Co.) on a gyrotory shaker at 37°. Scintillation vials were replaced at 30-min intervals and analyzed for radioactivity. The points represent accumulative values.

N1S1-67 cells only at 10-20% the rate observed with glucose (12).

Deoxyglucose uptake by N1S1-67 cells was inhibited by prednisolone (Fig. 2A), but approximately 50% less than the metabolism of glucose at comparable concentrations of substrates and inhibitor. The formation of all three intracellular intermediates was equally affected by prednisolone (Fig. 2B), supporting the conclusion that the transport of deoxyglucose was inhibited and not its further intracellular conversions. The effect on deoxyglucose transport was immediate and the degree of inhibition did not change during 8 hr of

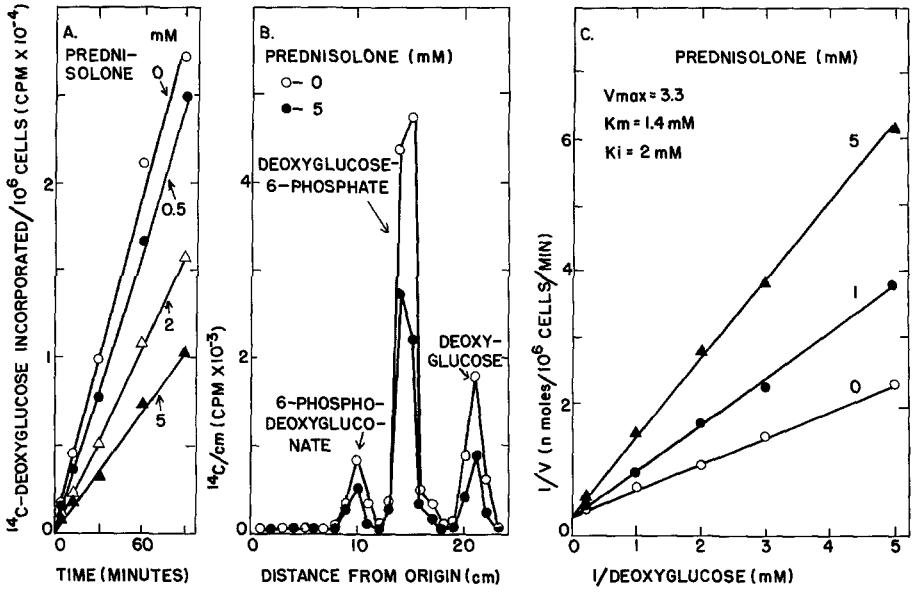

Fig. 2. Effect of prednisolone on deoxyglucose transport. (A and B) Samples of a suspension of 2 x 10^6 cells/ml of glucose-free BM42 were supplemented with prednisolone as indicated and immediately thereafter (0 time) with 30 μM ^{14}C-UL-2-deoxy-D-glucose (10 cpm/pmole). Duplicate 0.5-ml samples were analyzed for radioactivity into total cell material (A). After 90 min of incubation, acid-extracts were prepared from 1 x 10^7 cells and analyzed by ascending paper chromatography (B) with a solvent composed of 1 M ammonium acetate (pH 5.0) and 95% ethanol (3:7; v/v). After chromatography, the chromatograms were cut into 1-cm segments and these analyzed for radioactivity (11). (C) Portions of a suspension of 2 x 10^6 cells/ml of glucose-free BM42 were supplemented with the indicated concentrations of prednisolone and immediately thereafter 10-ml samples of each portion were supplemented with 0.2, 0.33, 0.5 or 1 mM ^{14}C-UL-2-deoxy-D-glucose (348 cpm/pmole) or with 4 mM ^{14}C-deoxyglucose (83 cpm/pmole). After 5, 10 and 20 min of incubation, duplicate 1-ml samples were analyzed for radioactivity in total cell material. The initial velocities were estimated from the linear portions of the incorporation curves (see A).

incubation of the cells with prednisolone either in the absence (Fig. 3) or presence of glucose (not shown). These results suggest that prednisolone acted directly on deoxyglucose transport. This conclusion is also indicated by the Lineweaver-Burk plots in Fig. 2C which show that deoxylgucose transport was inhibited by prednisolone in an apparent competitive manner.

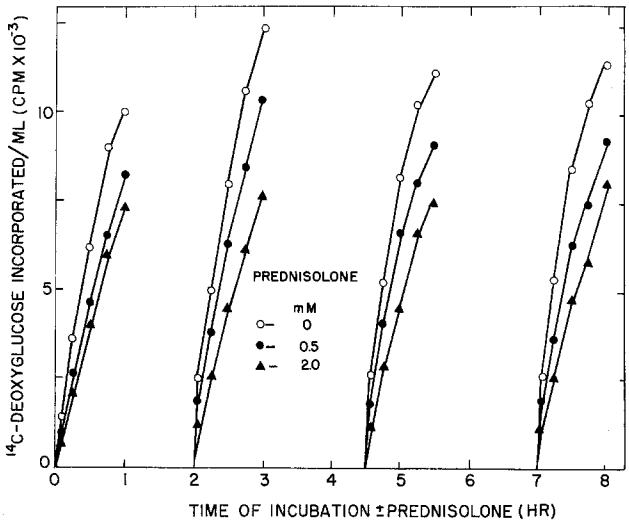

Fig. 3. Effect of prolonged incubation with prednisolone on deoxyglucose uptake. Portions of a suspension of 2 x 10^6 cells/ml of glucose-free BM42 were supplemented with the indicated concentrations of prednisolone (0 time). Immediately thereafter and at 2, 4.5 and 7 hr of further incubation, samples of each suspension were supplemented with 10 μM ^{14}C-deoxyglucose (10 cpm/pmole) and monitored for radioactivity incorporated into total cell material. All points represent averages of duplicate 1-ml samples.

It is not clear whether prednisolone inhibits glucose transport by specifically interacting with the glucose transport binding site or carrier protein or by blocking these sites due to a binding to hormone receptor sites in their close proximity. If the former is the case, the finding that the apparent Ki for prednisolone inhibition (2 mM) is similar to the Km for deoxyglucose transport (1.4 mM, Fig. 2C) suggests that prednisolone has about the same affinity for the deoxyglucose binding site as the substrate itself. The effect of prednisolone on glucose transport by N1S1-67 cells seems to be specific, since the transport of uridine, adenosine and choline by the cells (11, 14) was not affected by 5 mM prednisolone (not shown). However, the physiological significance of the competitive effect of hydrocortisone on glucose transport requires further elucidation. It seems possible that this represents the primary effect of glucocorticoids and that differences in the sensitivity of various cells

Table I. Tyrosine Aminotransferase Activity and Growth of NlSl-67 Cells in Presence of Prednisolone[1].

Glucose mM	Prednisolone mM	Cells/ml (10^{-6})	Tyrosine Aminotransferase milliunits/mg protein
0	0	2.15	0.54
0	1	2.10	0.55
15	0	3.20	0.55
15	1	3.15	0.57

[1]Cells were collected from an exponential phase culture and suspended to 1.4×10^6 cells/ml in glucose-free BM42. Samples of the suspension were supplemented with glucose and prednisolone as indicated and incubated on a gyrotory shaker at 37° for 15.5 hr. The suspensions were analyzed for cell concentration and cell-free extracts were assayed for tyrosine aminotransferase activity. One unit of activity was defined as the amount of enzyme producing 1 μmole of β-hydroxy-phenylpyruvate/min at 37°.

to glucocorticoids may reflect differences in the relative affinities of the glucose transport sites for substrate and glucocorticoids. For instance glucocorticoids cause cell death of mouse lymphoma cells (14) and cause the inhibition of glucose metabolism by cortisol-sensitive lymphosarcoma P1798 cells (6) at concentrations as low as 0.1 μM, while other isolates of the same lymphosarcoma are not affected by much higher concentrations (6). NlSl-67 are also highly resistant to glucocorticoids since the presence of 1 mM prednisolone had no effect on cell growth (Table I) and prolonged incubation of the cells with concentrations of 0.1 mM and below had no effect on glucose metabolism (not shown). Tyrosine aminotransferase induction also did not occur in NlSl-67 cells. The activity of the cells was low and not affected by incubation of the cells with 1 mM prednisolone for 15.5 hr either in the presence or absence of glucose (Table 1).

ACKNOWLEDGEMENTS

This work was supported by grants from the Minnesota Medical Foundation. We thank John Erbe and Lynn Scarborough for competent technical assistance.

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