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INHIBITION BY DEXAMETHASONE OF THE IN VITRO TRANSPORT OF 3-*O*-METHYLGLUCOSE INTO RAT THYMOCYTES

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Summary

Reduced glucose transport across the plasma membrane and reduced phosphorylation may both be responsible for the early inhibitory effect of physiological concentrations of glucocorticoids on glucose uptake by rat thymocytes.

The early inhibitory effects of glucocorticoids ($5 \cdot 10^{-7}$ M dexamethasone) on glucose consumption and $^{14}CO_2$ formation from D-[U-^{14}C]glucose were reproduced.

The total uptake curve of 4.8 μM 3-*O*-[^{14}C]methyl-D-glucose was biexponential with $t_{1/2}$ of 1.1 min and 36 min, respectively, the rapid part comprising about 50% of the equilibrated intracellular water space. The latency of the effect of $5 \cdot 10^{-7}$ M dexamethasone on 3-*O*-[^{14}C]methyl-D-glucose uptake ranged from 15 to 100 min and the inhibition varied from 15 to 55% independently of the lag period. The effect of 3-*O*-methylglucose concentration on the initial uptake by steroid-responsive cell preparations was tested after 45 min of preincubation with or without $5 \cdot 10^{-7}$ M dexamethasone. In 12 experiments dexamethasone reduced V from 1.36 ± 0.16 mmol $\cdot$ min$^{-1} \cdot$ l^{-1} cell water to 0.81 ± 0.10 mmol $\cdot$ min$^{-1} \cdot$ l^{-1} cell water with insignificant change of K_m (6.0 mM versus 5.9 mM). Dexamethasone had similar effect after 90 or 120 min.

The variabilities of control cell transport capacity, the lag period and the magnitude of the dexamethasone effect could not be accounted for by changes in pH, effects of cell density, concentrations of albumin, ethanol, nucleosides, pyruvate or correlated to age and sex of the rats. In conclusion the inhibition

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Abbreviations: Methylglucose, 3-*O*-methyl-D-glucose; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

of glucocorticoids on glucose consumption by thymocytes appears to be an inhibited plasma membrane transport capacity.

Introduction

The inhibitory effect of physiological concentrations of glucocorticoids on the in vitro uptake of glucose by rat thymocytes discovered by Munck and coworkers [1] has played a major role in the experimental approach to elucidate the mechanism of action of glucocorticoids. Firstly this effect is fairly rapid (15—25 min after steroid addition) and thus limits the early hormone-induced transcriptional and translational events to this short time period, secondly it could be a key metabolic effect for later effects [2]. This possibility is, however, negated by the observation that a compensatory load of glucose does not eliminate all later hormone effects [1].

The effect has been studied in detail by Munck and coworkers [1,3—6] and the reduced disappearance rate of glucose from the suspension medium of steroid-treated cells, the reduced cell content of Glc-6-*P* and lactate formation have readily been understood from an inhibition of the carrier-mediated glucose transport across the plasma membrane [2]. Direct support was obtained by data mentioned in abstract form on the inhibition by glucocorticoids of the transport of non-metabolisable glucose analogue 3-*O*-methylglucose [7,8]. During the completion of the present manuscript the full paper by Zyskowski and Munck appeared with a kinetic analysis of the glucocorticoid inhibition of methylglucose transport in rat thymocytes [9]. However, other investigators failed to verify any effect on methylglucose transport [10] and argued for an inhibitory effect on glucose phosphorylation.

The present study was carried out to clarify the subject. The data have demonstrated a variable decreased methylglucose uptake at 37°C appearing from 15 to 100 min after the addition of $5 \cdot 10^{-7}$ M dexamethasone caused by a 15—55% reduction of V without any change of K_m of the glucose carrier.

Materials and Methods

Radiochemicals were from the Radiochemical Centre, Amersham: [^{14}C]-Methylglucose (spec. act. 50—60 Ci/mol), L-[1-^{14}C]glucose (spec. act. 40—50 Ci/mol), D-[U-^{14}C]glucose (spec. act. 2.99 Ci/mol) and ^3H_2O. Dexamethasone (9α-fluoro-11β,17α,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione), *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) and bovine serum albumin (fraction V) were supplied by Sigma, Silicone oil AR 200 (*d* 1.04) from Serva, inosine and adenosine from Merck, Lumagel from Lumac, Glucoquant from Boehringer and incubation vessels V 521 from Radiometer.

Animals used were female specific pathogen-free Sprague-Dawley rats (Møllegaards Laboratory, Denmark) weighing 100—125 g. The rats were adrenalectomized and maintained at 0.9% NaCl, Rostock mixture ad libitum and at the diurnal light period. Animals were used from 1 to 6 days after adrenalectomy.

Rat thymocytes were prepared at room temperature. 1—4 rats were decapitated and their thymi immediately removed, placed in 10 ml buffer containing

Hank's salt solution (145 mM Na^+, 8.7 mM K^+, 0.8 mM Mg^{2+}, 1.3 mM Ca^{2+}, 142 mM Cl^-, 7.7 mM phosphate, 0.8 mM SO_4^{2-}, 4.1 mM HCO_3^-) supplemented with 0.3% (w/v) bovine serum albumin dialyzed twice against glass-distilled water, and 10 mM Hepes adjusted to pH 7.40 at 24°C. Adjacent connective tissue and lymph nodes were removed. In another 10 ml of buffer the thymi were minced with a scalpel. The cell suspension was decanted and washed three times by 8 min centrifugation at 300 × *g* removing the supernatant followed by careful resuspension of the cells. Finally the cell suspension was passed through a nylon net and adjusted to the proper cell concentration ranging from $3 \cdot 10^7$ to $5 \cdot 10^8$ cells/ml. Before use the cells were temperature equilibrated for 15 min.

Dexamethasone was added to the cells as an ethanolic solution giving a final dexamethasone concentration of $5 \cdot 10^{-7}$ M and an ethanol concentration of 0.07% (v/v). This amount of ethanol did neither change the control methylglucose transport levels nor the dexamethasone effect on this transport. The concentration of the stock steroid solution was checked by ultraviolet absorption at 239 nm (ϵ = 14 500).

Separation of the cells from the medium was carried out by oil centrifugation of 300-μl aliquots for 15 s in a Beckman Microfuge as described by Andreasen et al. [11] using 150 μl silicone oil but no dextran in plastic microtest tubes.

For methylglucose transport assay a stock cell suspension was labelled with ^3H_2O. 1 ml aliquots from this 37°C suspension were transferred in prewarmed constriction pipettes to 37°C transport vials containing 300 μl buffer with [^{14}C]methylglucose (final concentration 4.3 μM) and concentrations of unlabelled methylglucose ranging from 0 to 170 mM. Samples of twice 300 μl were removed at appropriate times for oil centrifugation. Termination of the transport was defined as the starting time of the microfuge. Immediately after pelleting 150 μl supernatant were removed for counting and the cell pellet recovered for counting by cutting the tip of the microtest tube by a pair of nippers. The tips were placed in scintillation vials, vigorously shaken and extracted with 5 ml Lumagel containing 3% H_2O at 37°C for 12 h. Before liquid scintillation counting the pellets were shaken again. No differences were detectable when counting results obtained after sonication of the pellet or digestion with 0.1 N NaOH were compared to results from the extraction procedure. Trapping of extracellular water was determined using 4 μM L-[1-^{14}C]glucose as described for methylglucose.

The results were computed by dividing the ratio of ^{14}C counts in the pellet and supernatant with the ratio of ^{3}H counts in the pellet and supernatant. This ratio represents the fractional equilibration of total pellet water space. Taking into account the trapped extracellular water space and the final equilibrated pellet water space for methylglucose determined after 240—300 min uptake of 4—5 μM this figure could be converted to fractional saturation of the final equilibrated intracellular water space.

Measurement of DNA was carried out on cell pellets according to the method of Burton [12].

Cells were sampled after preincubation or in parallel incubations for counting in a Neubauer haemacytometer, and the viability checked by the ability of

the cells to exclude 0.5% eosin added in Hank's salt solution. Viability generally exceeded 90% from start to ending of the experiments.

Liquid scintillation counting was performed in a Packard TriCarb liquid scintillation counter. The efficiency for ^{14}C was about 65%, for ^{3}H about 23% and the spillover from the ^{14}C to the ^{3}H channel amounted to about 16% and from ^{3}H to the ^{14}C channel 0.3%.

Results

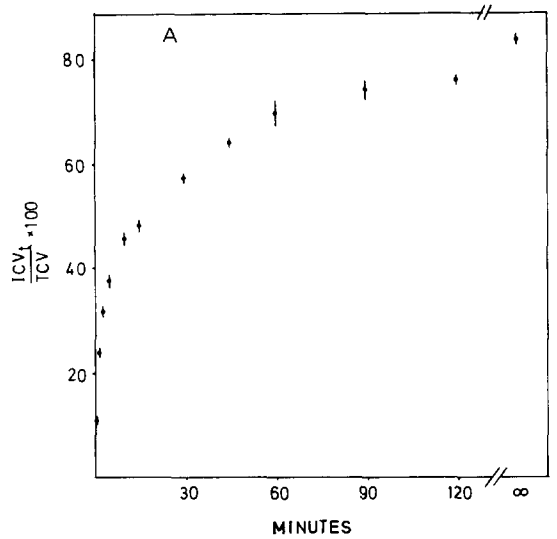
Thymocytes from adrenalectomized rats contained (mean ± S.E.) 8.4 ± 0.7 pg DNA per cell (n = 145) and the average cell water content amounted 104 ± 11 fl (n = 79).

The reactivity of thymocytes obtained by the present preparation procedure was tested in a few experiments in which an early 20—50% inhibition of glucose consumption (six experiments) and a 10—35% inhibition of $^{14}CO_2$ formation from D-[U-^{14}C]glucose (eight experiments) were reproduced (data not shown).

L-Glucose space measured at 30 or 90 s after addition of 4.0 μM L-[1-^{14}C]-glucose did not differ significantly and ranged from 12 to 16 % of total pellet water space and was not changed by dexamethasone. The final distribution volume for methylglucose determined after 240—300 min equilibration with 4—5 μM [^{14}C]methylglucose ranged from 82 to 90% of total pellet water space equivalent to 79—88% of total intracellular water space, and was not dependent of dexamethasone.

Investigations on the magnitude of a non-saturable part of methylglucose transport was investigated by following net uptake of L-[1-^{14}C]glucose at 4.0 μM at 37°C. After 15 min the change in L-glucose space was insignificant. Furthermore 170 mM methylglucose prevented the uptake of 4.3 μM [^{14}C]-methylglucose for 2.5 min. The precision of this observation was, however, lowered by the fact that the osmotic effect reduced the total pellet water space by 20—25% and correspondingly increased L-glucose space a little to 18%. When thymocytes were preloaded with 4.3 μM [^{14}C]methylglucose and subsequently diluted into a buffer containing 0.4 mM phloretin the efflux from the cells was prevented for 5 min at 37°C.

The uptake of 4.8 μM [^{14}C]methylglucose was followed to equilibrium (Fig. 1) and could be resolved in two exponential functions, a slow one ($t_{1/2}$ = 36 min) representing in this experiment 53% of the equilibrated intracellular water space and a rapid one ($t_{1/2}$ = 1.1 min) representing the rest. In five experiments the rapid phase ranged from 35 to 75% of the equilibrated intracellular water space. Further characterization of the initial uptake was obtained in experiments as given in Fig. 2. Control and dexamethasone-treated cells were preincubated for 45 min and [^{14}C]methylglucose uptake was measured from 12 to 120 s and at 240 min. The initial uptake was reduced by about 40% at 30 s and 30% at 120 s by dexamethasone. The initial uptake was almost linear for 40 s and might be extrapolated to zero time to the value of trapped extracellular water space as determined by L-glucose. The onset of dexamethasone inhibition of the early uptake of 4—5 μM [^{14}C]methylglucose varied from one preparation to another (Fig. 3).



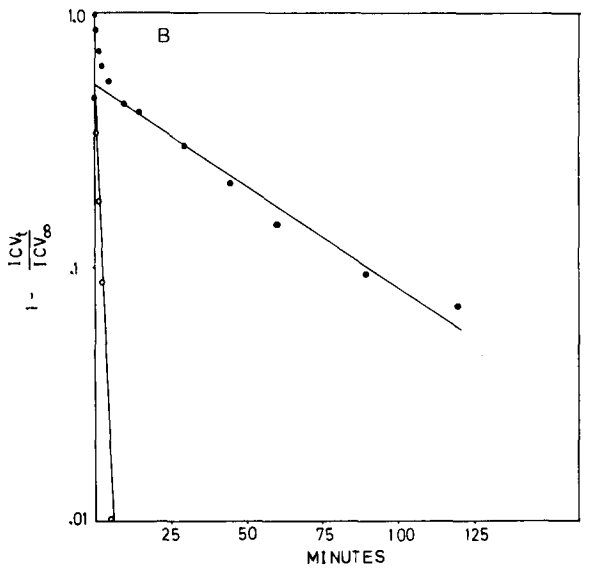


Fig. 1. Time course of the total uptake of [^{14}C]methylglucose by rat thymocytes. ^3H_2O-labelled thymocytes ($6.5 \cdot 10^7$ cells/ml) were incubated for 15 min at 37°C in two stirred vessels. At time zero [^{14}C]-methylglucose was added giving a final concentration of 4.8 μM. Twice 300 μl cell suspension were withdrawn from each vessel and oil centrifuged at the indicated times. The trapped extracellular water was estimated separately by L-glucose as described in Materials and Methods. It amounted 14% of total cell pellet water space. At apparent steady state the intracellular accumulated methylglucose represented less than 0.5% of the extracellular amount. (A) Shows the filling of the total intracellular water space. The bars represent S.E. (n = 4). The value at time zero represents mean of 210, 240 and 300-min values. Ordinate: percentage saturation of intracellular water space. ICV_t, intracellular water space equilibrated at time t. TCV, total intracellular water space. (B) the mean values in a semilogarithmic plot. Ordinate: 1-fractional equilibration of the intracellular distribution space for methylglucose. ICV_t, intracellular water space equilibrated at time t. ICV_∞, final intracellular distribution space at t = 210—300 min. Lines were fitted by eye. •, mean values; ○, values after subtraction of slow part.

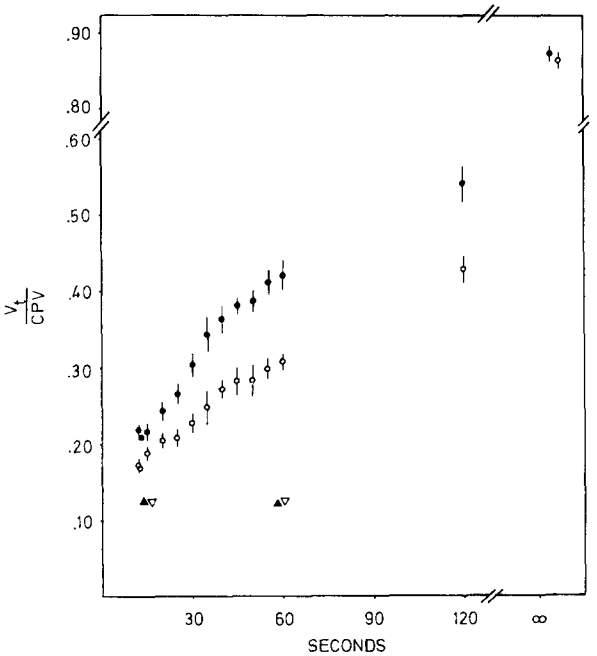


Fig. 2. The effect of dexamethasone on the early time course of the uptake of [^{14}C]methylglucose by rat thymocytes. Thymocytes (10^8 cells/ml) were preincubated in two stirred vessels with or without $5 \cdot 10^{-7}$ M dexamethasone at 37°C. Uptakes were determined at 4.3 μM [^{14}C]methylglucose and 4.0 μM L-[1-^{14}C]glucose. The procedure was as described in Materials and Methods but transport vials containing only 150 μl tracer and only 500 μl cell suspension were added. From each vial only one sample of 300 μl was oil centrifuged. Control and steroid-treated cells were tested alternatively. Each point was determined in quadruplicate. The value at infinite time was taken after 240 min. Symbols represent mean, bars are S.D. ●, control cells, methylglucose; ▲, control cells, L-glucose; ○, dexamethasone-treated cells, methylglucose; ▽, dexamethasone-treated cells, L-glucose. Ordinate: fractional saturation of total cell pellet water space. V_t, cell pellet water space equilibrated at time t. CPV, total cell pellet water space.

It was noticed that some control cell preparations showed increasing transport rate during the first 30 min after the end of temperature equilibration; others did not. The dexamethasone inhibition was significant between 20 and 50 min in preparations with increasing transport levels and between 40 and 100 min in preparations with constant transport levels. Two extremes in reactivity are illustrated in Fig. 3. Fig. 4 gives the frequency distribution of time periods necessary for a significant inhibition (15%) to occur. Maximal inhibition was established within 20 min after the onset and steroid inhibition was established in 60% of preparations after a lag period of less than 45 min.

A kinetic analysis of 30-s uptakes of methylglucose within a concentration range of 0.2—27 mM was performed after 45 min of preincubation with or without $5 \cdot 10^{-7}$ M dexamethasone. About 40% of preparations were eliminated from the analysis since the response was insufficient for the kinetic analysis. Fig. 5 shows a Hofstee plot from 12 experiments with steroid-responsive cell preparations. The composite data revealed identical K_m values for control cells (6 mM) and dexamethasone-treated cells (5.9 mM) whereas dexamethasone changed V from 1.36 mmol $\cdot$ min$^{-1} \cdot$ l^{-1} cell water to 0.82 mmol $\cdot$ min$^{-1} \cdot$ l^{-1} cell water. Each of the 12 selected experiments had identical K_m values for

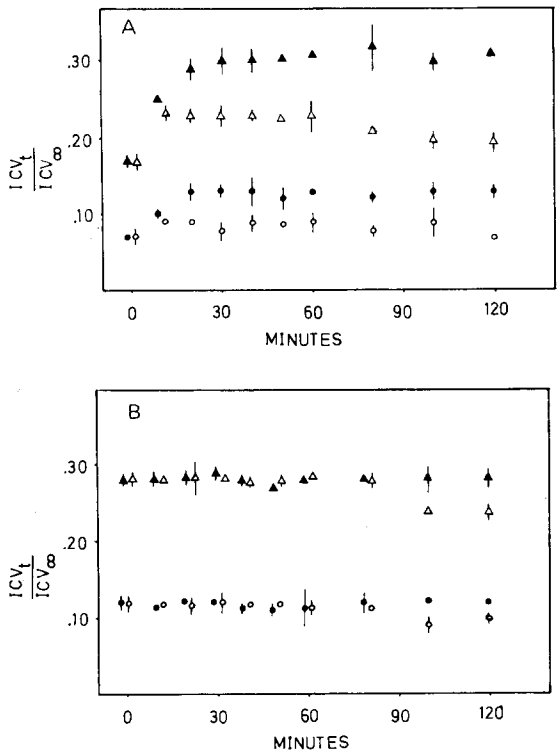


Fig. 3. Two extremes of the latency of the dexamethasone effect on methylglucose uptake. Thymocytes from three thymi were incubated with or without $5 \cdot 10^{-7}$ M dexamethasone at 37°C at time zero. At the indicated times after steroid addition the 30 and 90 s uptake of 4.2 μM [^{14}C]methylglucose or L-[^{14}C]-glucose were performed as described in Materials and Methods. The final distribution volume was tested after 240 min and was not significantly different for control and dexamethasone-treated cells. Ordinate: fractional saturation of the final intracellular distribution water space. ICV_t, intracellular waterspace equilibrated at time t. ICV_∞, final equilibrated intracellular water space measured after 240 min. ●, control cells, 30 s uptake; ▲, control cells, 90 s uptake; ○, dexamethasone-treated cells, 30 s uptake; △, dexamethasone treated cells, 90 s uptake. Bars represent range of double estimations. (A) Illustrates an early 30% inhibitory effect of dexamethasone on a cell preparation characterized by increasing control methylglucose transport levels. (B) Illustrates a late 15% inhibitory effect of dexamethasone on a cell preparation characterized by constant control methylglucose transport levels.

control and steroid-treated cells although the hormonal effect on V varied from 20 to 55%. From the 12 individual Hofstee plots the K_m values ±S.E. were 6.0 ± 0.5 mM and 5.9 ± 0.6 mM for control and dexamethasone-treated cells, respectively, and the corresponding V values 1.36 ± 0.16 and 0.81 ± 0.10 mmol · min^{-1} · l^{-1} cell water. The Hofstee plots testified again the absence of a non-saturable part of methylglucose transport up to at least four times K_m. Uptake analyses made after 90 or 120 min of preincubation with dexamethasone gave indistinguishable results. Assuming thymocytes to be regular spheres with a water content of 104 fl and a volume of 123 fl assuming a dry weight percentage of 21 and the mean cell density to be 1.07 [11] the maximal permeability could be calculated to be $3.2 \cdot 10^{-7}$ cm/s.

Several measures were taken to reduce the frequently observed increasing control transport capacity and the variability of the latency of the steroid inhibition. The pH remained constant throughout the experiments and chang-

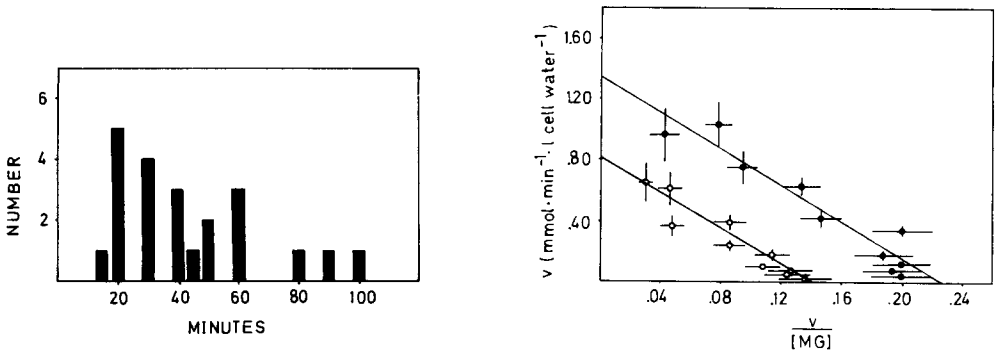


Fig. 4. Frequency distribution of the dexamethasone latency. 22 experiments as decribed in Fig. 3 and Materials and Methods were performed to investigate the latency of $5 \cdot 10^{-7}$ M dexamethasone to inhibit [^{14}C]methylglucose transport significantly (at least 15%) after 30 s uptake. Ordinate: number of experiments. Abscissa: lag period in minutes.

Fig. 5. Hofstee plot of the concentration dependence of the initial uptake of [^{14}C]methylglucose by control and dexamethasone-treated rat thymocytes. Cells ($3-8 \cdot 10^7$ cells/ml) were tested after 45 min of preincubation with or without $5 \cdot 10^{-7}$ M dexamethasone. 30-s uptakes of 4.3 μM [^{14}C]methylglucose at ten concentrations of unlabelled methylglucose ranging from 0.2 to 27 mM were performed as described in Materials and Methods. Data were corrected for trapped extracellular water as determined by parallel L-glucose space measurements. Transports were carried out in duplicate at each concentration level, alternatively testing control and dexamethasone-treated cells. Results are mean ± S.E. for 12 separate experiments. The vertical bars were calculated under the assumption that the error in methylglucose concentration was negligible. v, Initial uptake of methylglucose (mmol · min^{-1} · l^{-1} cell water). MG, concentration of methylglucose in transport medium (mM). •, control cells; ○, dexamethasone-treated cells. The lines were calculated by the method of least squares. Control cells showed K_m = 6.0 mM and V = 1.36 mmol · min^{-1} · l^{-1} cell water. Dexamethasone-treated cells showed K_m = 5.9 mM and V = 0.81 mmol · min^{-1} · l^{-1} cell water.

ing the buffer from Hepes to bicarbonate or phosphate made no difference. Neither bovine serum albumin nor ethanol were responsible for the control transport instability as the same phenomenon was observed in experiments without these compounds (dexamethasone dissolved in buffer). Neither cell concentrations ranging from $2 \cdot 10^7$ to $2 \cdot 10^8$ cells/ml nor supply of 5 mM sodium pyruvate as energy source made cell preparations more uniform. Addition of 1 mM adenosine or 1 mM inosine as substrate [13] resulted in either stimulation or inhibition of the initial uptake. The steroid effect was, however, superimposed with the usual variation in latency. 0.6 mM dibutyryl cyclic AMP did not antagonize the increase in control transport capacity and did not affect the steroid response. The steroid effect could be observed at 42°C during the incubation and transport assay, but experiments exceeding 60 min were not performed because of loss of cell viability. When the steroid effect was present at 37°C it was retained with the transports tested at 24°C (initial uptakes measured at 1 and 3 min). Thymocytes centrifuged through a 18/26/36% (w/v) discontinuous albumin gradient as described by Reeves [14] separated partially in two subpopulations one predominantly fast cells the other predominantly slow cells. The steroid responsiveness of the separated subpopulations was reduced compared to the unfractionated thymocytes. Time period after adrenalectomy, age, sex and seasonal variation did not correlate with transport stability or responsiveness.

Discussion

The rate of glucose transport across the plasma membrane is generally accepted as rate limiting for glucose consumption by rat thymocytes [15].

The kinetics of the rat thymocyte glucose carrier have been extensively studied using methylglucose by Reeves [14,16—18], Whitesell and coworkers [19—22] and Yasmeen et al. [15]. The carrier-mediated transport was found to be asymmetrical [20] and net uptake curves at concentrations far below K_m could be resolved into two exponential functions one with $t_{1/2}$ of 0.7—1.7 min, the other with $t_{1/2}$ of 12—35 min [14,21]. These figures have been explained by the existence of two populations of thymocytes representing small slow cells and larger quick ones [14,21]. This hypothesis was substantiated by differences in size distribution, uridine, thymidine and leucine incorporation by thymocyte suspensions separated on an albumin gradient [14] and further evidence was achieved by autoradiography of cells pulse-labelled with 2-deoxy[^{3}H]glucose [21]. The present data on biexponential uptake at low concentrations and separation of enriched subpopulations thus are in accordance with the concept of subpopulations. Kinetic constants for the fast methylglucose uptake by glucose-free thymocytes have been reported and are summarized in Table I. The present data are within the same range as those of Whitesell et al. [19] and Yasmeen et al. [15]. Reeves [16] used 1 min uptake values for initial uptake measurements which, compared to the reported $t_{1/2}$ for the rapid phase of 0.8—1 min, explains the higher K_m value; and the use of 2-min uptakes in Ref. 9 gave the same high K_m. Different methods for correction of trapped extracellular volume might account for the high V in Ref. 9 but another explanation could be anaerobic transport stimulation of the rather concentrated cell suspensions ($5 \cdot 10^8$—$2 \cdot 10^9$ cells/ml) used in that study. The slow cells were characterized by Whitesell and Regen [22] by a K_m four times higher and a V four times lower than for quick cells.

Present attempts to identify factors responsible for the spontaneous increase in control cell transport capacity in glucose-free media were not successful. The phenomenon also appears from the data in Refs. 9 and 19 and Reeves could partly prevent this stimulation by the addition of 10 mM DL-β-hydroxybutyrate [16]. Modulations of the glucose transport changing slow cells into fast

TABLE I

KINETIC CONSTANTS FOR INITIAL UPTAKE OF METHYLGLUCOSE BY RAT THYMOCYTES AT 37°C

V was calculated from the published data assuming 1 ml packed cell volume to contain $5 \cdot 10^9$ cells [7, 19], 1 mg protein to equal $5 \cdot 10^7$ cells [16] and one thymocyte to contain 104 fl water from the present study.

Reference	K_m (mM)	V (μmol/h per 10^{10} cells)
Reeves [15]	12.1	62.4
Whitesell et al. [18]	7.7	76.8
Yasmeen et al. [14]	4.4	57
Zyskowski et al. [9]	13.7	188
Present study	6.0	85

ones have been reported to correlate with enhanced intracellular Ca^{2+} [17,19] and lowering of ATP levels [16]. In the present study neither substrates such as pyruvate nor adenosine [13] prevented the increasing control transport. The increase in glucose transport may be an adaptation of some of the cells to the in vitro conditions. When operative if glucose is present the fast transport results mainly in lactate formation.

In contrast to the extensive studies on glucocorticoid effects on glucose metabolism in rat thymocytes (reviewed by Munck and collaborators [2,23]) few papers have dealt with direct characterisation of the postulated effect on the glucose transport. Makman et al. [24] were the first to report an inhibited uptake of 0.1 mM methylglucose 1—1.5 h after treatment of rat thymocytes with 10^{-6} M cortisol with 1.8 mM glucose present in the medium. The significance of the observation is questionable since a steroid-mediated inhibition of glucose uptake during the first hour would result in a higher glucose concentration in the medium and thus a competitive inhibition of the following 30 min uptake of methylglucose by steroid-treated cells. Furthermore no effect was found on methylglucose efflux.

Feldman et al. [10] failed to show an inhibitory effect on initial uptake of methylglucose at 25°C after 30 and 180 min of preincubation at 37°C with 10^{-6} M cortisol, although an inhibition of aminoisobutyric acid was evident after 180 min. A significant inhibitory effect on the uptake of aminoisobutyric acid accumulating for a 5 min period at 37°C would be more easily observed than small inhibitory effects on the equilibrating methylglucose transport measured during 1.5 min at 25°C. Thus the indicated standard errors for the three experiments presented cannot exclude a 10—15% effect. Even with the present technique in which the volume of trapped medium was much smaller a 10—15% inhibition might escape detection after 30 s at 37°C corresponding to 1.5 min at 25°C. Finally the validity of the kinetic analysis of thymocyte methylglucose transport in the paper of Feldman et al. [10] must be questioned. About 30% of the transport seemed to be non-saturable at 25°C but in the same paper glucose inhibited methylglucose uptake by 95% and so did 1 μg/ml cytochalasin which in fat cells have been used to assess the non-saturable transport [25]. With the oil centrifugation method it was found that the inhibitory effect on methylglucose uptake produced at 37°C was retained at 24°C.

The conclusion of Zyskowski and Munck's work on rat thymocytes [9] was that 10^{-6} M cortisol significantly decreased V for transport of methylglucose with only marginal changes on K_m. This was supported by the kinetic analyses at 23°C, 41°C and after 25 min of cortisol treatment at 37°C. However, after 60 min of cortisol treatment the kinetic analysis at 37°C came out with a V reduced by 46% and a K_m reduced by 52%. If this was true one would expect a slight stimulation by cortisol of the initial uptake of methylglucose at concentrations far below K_m. However, an inhibition by 20—30% was documented in Figs. 1—3 in Ref. 9. Consequently that particular part of the kinetic analysis must be questioned.

In fat cells Livingston and Lockwood [26] have demonstrated an inhibition of glucose uptake at 37°C by 10^{-7} M dexamethasone after 30 min with maximal effect after 1—2 h. V decreased by about 40% without any changes in K_m.

An inhibitory effect on 0.1 mM methylglucose uptake after 2 h with 10^{-7} M dexamethasone tested at 21°C was reported without further kinetic data. Recently Foley et al. [27] found a 45% reduced D-arabinose uptake in fat cells treated with 10^{-6} M dexamethasone for 2 h.

From the present study it is evident that dexamethasone at a concentration sufficient within 1.5 min at 37°C to saturate the glucocorticoid receptor of rat thymocytes (K_d 6—8 nM [28], confirmed in this laboratory) exerts an inhibitory effect on the glucose carrier without a concomitant change of K_m. It should be mentioned that an inhibition of methylglucose influx in the present system reflects an effect on rapidly transporting cells. However, glucose transport is rate limiting for glucose metabolism in thymocytes indicating that also effects on glucose consumption and lactate formation predominantly reflect effects on these rapidly transporting cells.

The shortest lag period of the transport effect reported here might well explain the observed dexamethasone inhibition of glucose uptake and $^{14}CO_2$ formation and the early effects on glucose consumption, Glc-6-*P* levels and lactate production reported by Munck and coworkers [1,3—6]. Effects on glucose metabolism not easily explained by transport inhibition alone were reported in the data of Boyett and Hofert [29] on thymocytes. Cortisol increased during the first hour of incubation the specific yield of CO_2 from glucose indicating a chanelling of glucose metabolism towards oxidative phosphorylation. Roth and Livingston [30] demonstrated a loss of dexamethasone inhibition on transport but a persistent metabolic inhibition in adipocytes from old rats caused by decreased phosphorylation. The sensitivity of their transport assay might, however, be questioned, because transport by 12-month-old rats was reported to be stimulated 10—20% by dexamethasone.

Although simultaneous measurements of the time course and magnitude of the transport effect and the metabolic effects were not performed in the present study the rather long lag periods observed with some preparations (30—100 min) are probably not incidental but might reflect a decreased nuclear binding of the steroid-receptor complex observed in glucose-free medium [31] or minor changes in the adenine nucleotide balance known to correlate with reduced steroid inhibition of the RNA and protein synthesis [32,33].

In conclusion it seems most likely from the studies of Munck and coworkers and the present results that transport inhibition is the predominant reason for the inhibitory effects of glucocorticoids on glucose uptake by rat thymocytes in vitro.

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