Dexamethasone inhibits the hexose monophosphate shunt in activated rat peritoneal macrophages by reducing hexokinase-dependent sugar uptake

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Dexamethasone decreases 2-D-deoxyglucose (2-dGlc) uptake and accumulation into rat peritoneal macrophages *in vitro* in a concentration- and time-dependent manner (K_i for 1 μM -dexamethasone after a 2 h exposure = $0.71 \pm 0.21 \mu\text{M}$; K_i for 0.1 μM -dexamethasone after exposure for 4 h = $0.10 \pm 0.06 \mu\text{M}$). The inhibition of 2-dGlc uptake is consistent with a decrease in the coupling between endofacial hexokinase activity and the sugar transporter. The evidence for this is: (1) the K_m for *zero-trans* 2-dGlc uptake in quiescent macrophages was increased by dexamethasone, but there was no significant effect on the V_{max} ; (2) dexamethasone increased the rate of exit of sugar from cells preloaded with 2-dGlc; (3) the free sugar accumulation within the cytosol of the cells above the external solution concentration was significantly decreased by dexamethasone. These effects of dexamethasone on 2-dGlc transport were antagonized by simultaneous exposure to the steroid RU 38486 ($K_i = 0.04 \pm 0.01 \mu\text{M}$; 4 h incubation). Although dexamethasone inhibited *zero-trans* uptake, the maximum rate of *infinite-trans* exchange uptake of 2-dGlc into cells preloaded with 3-O-methyl-D-glucose (40 mM) was unaltered by dexamethasone or RU 38486, indicating that the dexamethasone-dependent decrease in *zero-trans* uptake was not due to a change in the number of transporters in the plasma membrane. Dexamethasone also inhibited the phorbol myristate acetate-induced stimulation of hexose monophosphate shunt (HMPS) activity, and this was reversed by RU 38486. Cytochalasin B, the potent sugar-transport inhibitor, inhibited HMPS activity and 2-d[2,6- ^3H]Glc uptake equally, indicating a single site of action. By contrast, dexamethasone showed differential inhibition of HMPS activity and 2-d[2,6- ^3H]Glc uptake, suggesting that it not only acts by decreasing the coupling between hexokinase and sugar transport, but also at one or more additional points.

INTRODUCTION

Glucocorticoids inhibit sugar transport in rat adipocytes (Carter-Su & Okamoto, 1985), human erythrocytes (Lacko *et al.*, 1975), chick-embryo fibroblasts (Rubin, 1977), rat thymocytes (Morita & Munck, 1964; Makman *et al.*, 1971; Hallahan *et al.*, 1973) and murine peritoneal macrophages (Norton & Munck, 1980). The conventional theory used to explain these findings is that glucocorticoids selectively decrease the number of glucose transporters in the plasma membrane (Carter-Su & Okamoto, 1985). However, recent evidence from work on rat thymocytes suggests that in this cell type at least, dexamethasone, a synthetic glucocorticoid, inhibits 2-D-deoxyglucose (2-dGlc) uptake in a way that is consistent with decreased endofacial hexokinase activity in the vicinity of the glucose transporter (Naftalin & Rist, 1990). The object of the present study is to investigate if the inhibition of 2-dGlc uptake by dexamethasone in rat peritoneal macrophages can also be explained by decreased hexokinase activity.

In addition to their effects on sugar transport, glucocorticoids are known to modulate certain monocyte and macrophage functions (Guyre & Munck, 1987). For example, glucocorticoids inhibit the tumoricidal activity of murine macrophages (Hogan & Vogel, 1988) and the anti-microbial activity of human macrophages (Schaffner, 1985), and these effects may, at least in part, be due to a corticosteroid-induced suppression of superoxide production (Schultz *et al.*, 1985; Maridonneau-Parini *et al.*, 1989). Superoxide production by phagocytes is associated with a rapid increase in metabolism known as the 'respiratory burst',

and one of the characteristics of this process is increased hexose monophosphate shunt (HMPS) activity (Arthur *et al.*, 1986, 1988), which produces NADPH. NADPH is the substrate required by NADPH oxidase for the production of superoxide, and thus inhibition of the activity of the HMPS will lead to suppressed free-radical generation. In this paper, we used phorbol myristate acetate (PMA)-induced HMPS activity as an indicator of superoxide production in order to investigate the ability of dexamethasone to inhibit these processes.

RU 38486 is a steroid which has been shown to antagonize many of the effects of dexamethasone *in vivo* and *in vitro*, without marked agonist activity (Philibert, 1984). It has been suggested that RU 38486 inhibits glucocorticoid effects by binding to the cytosolic glucocorticoid receptor, thus impairing its activation (Moguilewsky & Philibert, 1984). However, the effect of RU 38486 on the dexamethasone-induced inhibition of 2-dGlc uptake in rat peritoneal macrophages has not previously been reported.

MATERIALS AND METHODS

Cell preparation

Rat peritoneal macrophages were prepared by the method described previously (Rist *et al.*, 1990). Anaesthetized Wistar rats (200–250 g body wt.) were killed by cervical dislocation and immediately, 25 ml of Hepes-buffered saline (Hepes, 5 mM; NaCl, 140 mM; KCl, 5 mM; MgCl₂, 1.2 mM), pH 7.3 at 37 °C, containing 0.85 % (w/v) NH₄Cl was injected into the peritoneal cavity of the rats. Macrophages were harvested by massage of the abdomen

Abbreviations used: 2-dGlc, 2-D-deoxyglucose; 3-OMG, 3-O-methyl-D-glucose; PMA, phorbol myristate acetate; HMPS, hexose monophosphate shunt.

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for 5–10 min, the cell suspension was then drawn out and centrifuged (300 *g* for 3 min), before being washed twice with saline. The average yield from one animal was approx. $(1-2) \times 10^7$ macrophages, and each experiment required three or four rats.

Microscopy and cell counting

Optical microscopy of the cell preparation showed intact cells, with little debris, and 95% of the cells excluded Evan's Blue stain. Differential counts of stained peritoneal washings showed > 90% macrophages and monocytes.

Estimation of the mean cell volume of the macrophages was performed by measurement of the 3-*O*-methyl-D-glucose (3-OMG) space and confirmed by using an Elzone 280PC particle analyser (Particle Data Ltd., Kingstone, Hereford, U.K.). Macrophage volume was estimated as 330 fl, and all sugar uptake rates (etc.) are normalized to 3×10^9 cells or ~ 1 ml of cell water. The Elzone 280PC analyser used to measure cell numbers, and macrophage suspensions were diluted to between 5×10^5 and 1×10^6 cells/ml.

Radioactive-sugar studies

Sugar uptakes into macrophages were performed using 2-deoxy-D-[2,6- ^3H]glucose or 2-deoxy-D-[1- ^3H]-glucose, (Amersham International; initial sp. radioactivity 42.5 Ci/mmol or 9 Ci/mmol respectively; final concn. approx. 0.5 $\mu\text{Ci}/\text{ml}$), with or without dexamethasone (Sigma) or RU 38486 (Roussel Labs). Sugar uptake and exit was stopped by adding iso-osmotic ice-cold saline containing phloretin and HgCl_2 at final concentrations of 100 μM and 1 μM respectively. Uptake in the presence of this 'stopping solution' gives the background passive uptake of sugar, and all uptake rates are expressed as phloretin-sensitive uptakes. After adding stopping solution, the cells were washed twice by centrifugation (for 3 min at 3000 *g*) and resuspension in fresh ice-cold stopping solution to remove extracellular isotope (a sample of the supernatant being retained for the determination of the extracellular radioactivity). Finally, the cells were lysed by vortex-mixing in distilled water, the lysate used to determine total uptake and, when required, free sugar and hexose phosphate concentrations within the cell pellet. Radioactivity was counted using scintillation fluid of the following composition: 1 litre of toluene (BDH), 1 litre of Synperonic NX (Durham Chemicals Distributors Ltd., Birtley, Tyne and Wear, U.K.) and 5 g of 2,5-diphenyloxazole (Sigma).

Estimation of phosphorylated and non-phosphorylated sugar

Separation of phosphorylated and non-phosphorylated sugar was performed by anion-exchange chromatography as previously described (Naftalin & Rist, 1989). Briefly, a sample of ice-cold cell lysate was filtered through a Whatman DE81 anion-exchange filter in a Buchner filtration tube and the first filtrate, containing free sugar, was sampled. The sugar phosphate retained on the filter was washed off with 0.5 M-HCl and also sampled. Both samples were then counted for radioactivity, and the amount of free sugar and hexose phosphate per 3×10^9 cells was estimated after correction for quench and dilution.

Radioactive sugar exit from preloaded cell suspensions

Freshly isolated macrophages were incubated with 2-d[1- ^3H]Glc (0.1 mM) for 4 h at 37 °C, with or without dexamethasone (1 μM). The macrophages were then centrifuged at 1000 *g* for 3 min, the supernatants removed and the cells washed in saline at 20 °C, before being re-centrifuged and finally resuspended in fresh saline with nominally zero sugar at 37 °C (2-dGlc < 0.005 mM).

Zero-trans net exit of total sugar was measured by observing

the time-dependent loss of radioactivity from the cells into the nominally sugar-free solutions.

Determination of HMPS activity

Macrophages have an active HMPS pathway (Newsholme *et al.*, 1986, 1987) that metabolizes 2-dGlc-6-phosphate via glucose-6-phosphate dehydrogenase and in the process cleaves the C-1 carbon atom from the sugar molecule. It follows that ^3H label on the C-1 carbon of 2-dGlc is lost from the cell as $^3\text{H}_2\text{O}$, whereas label at other positions will remain in the cytosol. Thus, by measuring the differential uptake of isotopes labelled at different positions, it is possible to determine the activity of the HMPS under various conditions.

RESULTS

Concentrations-dependent effects of dexamethasone and RU 38486 on 2-d[2,6- ^3H]Glc uptake into macrophages

Fig. 1 shows the concentration-dependence for dexamethasone on 2-d[2,6- ^3H]Glc (0.1 mM) uptake with or without RU 38486 (1 μM). Dexamethasone significantly decreased the uptake of 2-d[2,6- ^3H]Glc (0.1 mM) at all concentrations of glucocorticoid, ($P < 0.001$; in all cases $n = 6$) and the effect was concentration-dependent. The presence of RU 38486 (1 μM) during the preincubation significantly increased the uptake of 2-d[2,6- ^3H]Glc (0.1 mM) at 0, 0.1 and 1 μM -dexamethasone ($P < 0.05$, $P < 0.001$ and $P < 0.01$ respectively; $n = 6$), but did not have a significant effect at 10 μM -dexamethasone.

Fig. 2 shows the concentration-dependence for RU 38486 on 2-d[2,6- ^3H]Glc (0.1 mM) uptake in the presence or absence of dexamethasone (0.1 μM). RU 38486 caused a small, but significant, increase in sugar uptake at 0.01, 0.1 and 1 μM ($P < 0.05$, $P < 0.01$ and $P < 0.05$ respectively; $n = 6$). With dexamethasone (0.1 μM) present the uptake of 2-dGlc was significantly decreased at 0, 0.01 and 0.1 μM -RU 38486 ($P < 0.001$, in all cases; $n = 6$), but not at 1 μM -RU 38486.

These data indicate that dexamethasone causes a concentration-dependent inhibition of 2-dGlc uptake in native rat peritoneal macrophages, and RU 38486 competitively

Fig. 1. Concentration-dependent effects of dexamethasone on the uptake of 2-d[2,6- ^3H]Glc (0.1 mM) into macrophages with or without RU 38486 (1 μM)

Results are expressed as means $\pm$ S.E.M., corrected for phloretin-insensitive uptake. Macrophages were preincubated for 4 h at 37 °C in various concentrations of dexamethasone (0–10 μM) in the presence or absence of RU 38486 (1 μM). The cells were washed in saline, and uptake of 2-d[2,6- ^3H]Glc (0.1 mM) was then measured over a 15 min period. Dexamethasone significantly decreased the uptake of 2-dGlc at all concentrations ($P < 0.001$, in all cases; $n = 6$), and RU 38486 significantly increased uptake at 0, 0.1 and 1 μM ($P < 0.05$, $P < 0.001$ and $P < 0.01$ respectively; $n = 6$), but not at 10 μM -dexamethasone. □, Control; ■, RU 38486 (1 μM).

Fig. 2. Concentration-dependent effects of RU 38486 on the uptake of 2-d[2,6- ^3H]Glc (0.1 mM) into macrophages with or without dexamethasone (0.1 μM).

Macrophages were incubated at 37 °C for 4 h in various concentrations of RU 38486 (0–1 μM) and with or without dexamethasone (0.1 μM). The cells were then washed in saline before being incubated in 2-d[2,6- ^3H]Glc (0.1 mM) for 15 min. RU 38486 significantly increased the uptake of 2-dGlc at all concentrations ($P < 0.05$ at 0.01 and 1 μM ; $P < 0.01$ at 0.1 μM ; $n = 6$) and dexamethasone (0.1 μM) significantly decreased uptake at 0, 0.01 and 0.1 μM ($P < 0.001$, in all cases; $n = 6$), but not at 1 μM -RU 38486. Results are expressed as means $\pm$ S.E.M. $\square$, Control; $\blacksquare$, dexamethasone (0.1 μM).

Fig. 3. Time-dependent effects of dexamethasone (0.1 μM) on 2-d[2,6- ^3H]Glc (0.1 mM) uptake

Macrophages were incubated at 37 °C for various time periods (0–6 h) with or without dexamethasone (0.1 μM), washed in saline and then 2-d[2,6- ^3H]Glc (0.1 mM) uptake measured for 15 min. There was no significant effect of preincubation in the control cells, but dexamethasone caused a significant time-dependent decrease in the uptake of 2-dGlc, ($P < 0.001$, at all times; $n = 6$). $\square$, Control; $\blacksquare$, dexamethasone (0.1 μM).

antagonizes this effect. By contrast, RU 38486 alone has a small stimulatory effect on the uptake of 2-dGlc, this may result from inhibition of the effects of endogenous glucocorticoids.

Time-dependent effects of dexamethasone (0.1 μM) on 2-dGlc uptake

Fig. 3 shows that dexamethasone (0.1 μM) significantly decreased the uptake of 2-d[2,6- ^3H]Glc (0.1 mM) at 2, 4 and 6 h when compared with control ($P < 0.001$, in all cases; $n = 6$), and the inhibition of 2-dGlc uptake progressively increased as the preincubation time was increased. A 2 h preincubation with dexamethasone (0.1 μM) inhibited 2-dGlc uptake by approx. 25%, but after 6 h the inhibition was > 38%.

A time-dependent inhibition of 2-dGlc uptake by dexamethasone has been reported in human fibroblasts (Horner *et al.*, 1987).

Table 1. Effects of dexamethasone (Dexa) (0.1 μM) and RU 38486 (RU) (1 μM) on steady-state accumulation of free 2-d[1- ^3H]Glc

Cells were incubated in 2-d[1- ^3H]Glc (0.1 mM) for 2 h in the presence or absence of dexamethasone (0.1 μM) and RU 38486 (1 μM), and the free sugar and sugar phosphates were separated by anion-exchange chromatography. The free 2-dGlc accumulation was significantly decreased by dexamethasone ($P < 0.05$; $n = 6$), but with RU 38486 alone and RU 38486 with dexamethasone, accumulation of free sugar was not significantly different from the control. Results are expressed as means $\pm$ S.E.M.; * $P < 0.05$; n.s., not significant (test versus control).

Conditions	n	Free 2-dGlc accumulation ($\mu\text{mol} \cdot 2 \text{ h}^{-1} \cdot \text{ml of cell water}^{-1}$)
Control	8	2.20 $\pm$ 0.09
Dexa	6	1.94 $\pm$ 0.06*
RU	8	2.26 $\pm$ 0.06 ^{n.s.}
Dexa + RU	7	2.36 $\pm$ 0.13 ^{n.s.}

Effects of dexamethasone and RU38486 on steady-state accumulation of 2-d[1- ^3H]Glc

Macrophages were incubated for 2 h in 2-d[1- ^3H]Glc (0.1 mM) with or without dexamethasone (100 nM) and RU 38486 (1 μM), and the free and phosphorylated sugars were separated by anion-exchange chromatography (see the Materials and methods section). In control macrophages there was a 22-fold higher concentration of free 2-dGlc in the cytosol (2.20 $\pm$ 0.09 mM; $n = 8$), than in the external saline (0.1 mM; Table 1). This free-sugar accumulation was significantly decreased by dexamethasone (100 nM) to 19-fold (1.94 $\pm$ 0.06 mM; $n = 6$; $P < 0.05$). RU 38486 (1 μM) alone did not significantly alter the accumulation of free 2-dGlc (2.26 $\pm$ 0.06 mM, $n = 8$), but in combination with dexamethasone (100 nM) it reversed the inhibitory effect of the glucocorticoid such that free 2-dGlc accumulation was not significantly different from the control value (2.36 $\pm$ 0.13 mM, $n = 7$).

Effects of dexamethasone and RU 38486 on the zero-trans influx kinetics of 2-d[2,6- ^3H]Glc uptake

Total uptake of 2-d[2,6- ^3H]Glc was measured for 10 min at 37 °C over a range of concentrations of the sugar (0.01–10 mM) after preincubation for 2 or 4 h in the presence or absence of dexamethasone (0–1 μM) and RU 38486 (1 μM). The data obtained after a 2 h incubation with 1 μM -dexamethasone were plotted by non-linear least-squares regression using the ENZFITTER program of the Michaelis–Menten equation (Fig. 4, main diagram). In the control condition, the K_m for zero-trans uptake of 2-d[2,6- ^3H]Glc was 0.75 $\pm$ 0.13 mM and the V_{max} was 1.30 $\pm$ 0.06 $\mu\text{mol} \cdot \text{min}^{-1} \cdot 3 \times 10^9 \text{ cells}^{-1}$ ($n = 8$). Dexamethasone (1 μM) treatment for 2 h significantly increased the K_m for zero-trans uptake of 2-d[2,6- ^3H]Glc to 1.71 $\pm$ 0.14 mM ($P < 0.001$), without significantly altering the V_{max} (1.53 $\pm$ 0.11 $\mu\text{mol} \cdot \text{min}^{-1} \cdot 3 \times 10^9 \text{ cells}^{-1}$; $n = 6$).

By using the ENZFITTER program the K_m and K_p (i.e. the K_m in the presence of the inhibitor) were found for different concentrations of dexamethasone and RU 38486 over various time courses (results not shown) and then the K_i calculated from the formula:

$$K_i = [\text{inhibitor}] / [(K_p/K_m) - 1]$$

The data give a K_i of 0.71 $\pm$ 0.21 μM for the dexamethasone-dependent inhibition (1 μM) of zero-trans 2-d[2,6- ^3H]Glc uptake after a 2 h incubation. Preincubation for 2 h with 0.25 or 0.1 μM -dexamethasone gave K_i values of 0.72 $\pm$ 0.25 and 0.76 $\pm$ 0.19 μM

Fig. 4. Effects of dexamethasone (1 μ M) on the zero-trans influx kinetics of 2-d[2,6- 3 H]Glc uptake

The uptake of a range of concentrations of 2-d[2,6- 3 H]Glc (0.01 to 5 mM) into macrophages was measured for 10 min at 37 °C after preincubation for 2 h with or without dexamethasone (1 μ M). The results are expressed as means $\pm$ S.E.M. for phloretin-sensitive uptake and plotted by non-linear least-squares regression using the ENZFITTER program of the Michaelis-Menten equation (main diagram). In control cells the K_m for zero-trans uptake was 0.75 ± 0.13 mM and the V_{max} was $1.30 \pm 0.06 \mu\text{mol} \cdot \text{min}^{-1} \cdot 3 \times 10^9 \text{ cells}^{-1}$ ($n = 8$). Dexamethasone (1 μ M) significantly increased the K_m (1.71 ± 0.14 mM; $P < 0.001$), but did not significantly alter the V_{max} ($1.53 \pm 0.11 \mu\text{mol} \cdot \text{min}^{-1} \cdot 3 \times 10^9 \text{ cells}^{-1}$; $n = 6$). The inset shows a Lineweaver-Burk plot of the data, with the lines for control and dexamethasone both intersecting on the y-axis. This result indicates that dexamethasone is a competitive inhibitor of the zero-trans uptake of 2-d[2,6- 3 H]Glc. $\square$, Control; $\blacksquare$, dexamethasone (1 μ M).

respectively, and these were not significantly different from the K_i for 1 μ M. In contrast, preincubation for 4 h with 0.1 μ M-dexamethasone significantly decreased the K_i for dexamethasone-dependent inhibition of zero-trans 2-dGlc uptake to $0.10 \pm 0.06 \mu\text{M}$ ($P < 0.001$; $n = 5$), indicating that there is a time-dependent increase in the apparent affinity of agonist for the glucocorticoid-receptor-dependent inhibition of 2-dGlc uptake. The calculated K_i for RU 38486 (1 μ M) on the reversal of 2-dGlc uptake inhibition by a 4 h incubation with dexamethasone (1 μ M) was $0.04 \pm 0.01 \mu\text{M}$.

The data were also plotted as a Lineweaver-Burk plot (Fig. 4, inset), and this plot shows that the lines for control and dexamethasone intersect the y-axis at the same point, indicating that dexamethasone inhibits the uptake of 2-d[2,6- 3 H]Glc competitively.

Effects of dexamethasone (1 μ M) on the zero-trans net exit of 2-d[1- 3 H]Glc

The exit of sugar from macrophages preloaded for 4 h with 2-d[1- 3 H]Glc (0.1 mM) in the presence or absence of dexamethasone (1 μ M) was monitored by measuring the loss of radioactivity from the cells in nominally sugar-free medium (see the Materials and methods section). The decrease in total sugar

Fig. 5. Effect of dexamethasone (1 μ M) on the zero-trans net exit of 2-d[1- 3 H]Glc

Results (means $\pm$ S.E.M.) are expressed as the percentage loss of radioactivity with the zero-time value as the 100 % reference. The gradient for the exit of 2-d[1- 3 H]Glc (0.1 mM) from control cells was $-0.011 \pm 0.0011 \text{ min}^{-1}$ ($n = 5$) and was increased significantly by dexamethasone to $-0.015 \pm 0.0009 \text{ min}^{-1}$ ($n = 5$; $P < 0.05$). $\square$, Control; $\blacksquare$, dexamethasone (1 μ M)

is expressed as the percentage loss of radioactivity, with the zero-time value as the 100 % reference value. Fig. 5 shows that dexamethasone (1 μ M) significantly increased the rate of 2-d[1- 3 H]Glc (0.1 mM) exit from macrophages. The gradient for exit increased from $-0.011 \pm 0.001 \text{ min}^{-1}$ (control, $n = 5$) to $-0.015 \pm 0.001 \text{ min}^{-1}$ ($n = 5$; $P < 0.05$). Dexamethasone was reported to have the same effect on rat thymocytes (Naftalin & Rist, 1990).

Effects of dexamethasone (0.1 μ M) and RU 38486 (1 mM) on infinite-trans exchange uptake of 2-dGlc

Freshly isolated macrophages were incubated at 37 °C for 4 h with or without 40 mM-3-O-methyl-D-glucose (3-OMG), dexamethasone (0.1 μ M) and RU 38486 (1 μ M). The cells were then centrifuged, washed with ice-cold saline to remove extracellular 3-OMG and immediately the uptake of 2-d[2,6- 3 H]Glc (0.1 mM) was measured over a 5 min period at 37 °C (Table 2). Pre-loading with 3-OMG (40 mM) significantly increased the uptake of 2-d[(2,6- 3 H]Glc (0.1 mM) in all the conditions when compared with zero-trans uptake ($P < 0.001$, in all cases; $n = 6$), but there is no significant difference between the maximal rates of exchange uptake of 2-dGlc. Since dexamethasone (0.1 μ M) significantly decreased zero-trans uptake of 2-dGlc ($P < 0.01$; $n = 6$), it follows that the exchange component of 2-dGlc uptake into dexamethasone-treated cells pre-loaded with 3-OMG (40 mM) was increased. RU 38486 (1 μ M) alone did not significantly affect the zero-trans uptake of 2-d[2,6- 3 H]Glc (0.1 mM), but when present with dexamethasone it reversed the inhibitory effect of the glucocorticoid, such that zero-trans uptake was not significantly different from the control value. These findings are consistent with the view that dexamethasone reduces the coupling of hexokinase to 2-dGlc transport activity without changing the number of glucose transporters in the plasma membrane and that RU 38486 antagonizes these effects.

Effects of dexamethasone and RU 38486 on PMA-induced HMPS activity

Freshly isolated macrophages were incubated at 37 °C for 4 h with various concentrations of dexamethasone (0–10 μ M) with or

Table 2. Effects of dexamethasone (Dexa) (0.1 μM) and RU 38486 (1 μM) on the infinite-trans exchange uptake of 2-d [2,6-³H]Glc

Macrophages were preloaded with or without 3-OMG (40 mM) for 4 h at 37 °C in the presence or absence of dexamethasone (0.1 μM) and RU 38486 (1 μM). The cells were then washed with ice-cold saline (to remove the external sugar) and the uptake of 2-d[2,6-³H]Glc (0.1 mM) was measured over a 5 min period. Dexamethasone (0.1 μM) significantly decreased the zero-trans uptake of 2-dGlc (*P* < 0.01, *n* = 6), but RU 38486 (1 μM) alone did not significantly alter the uptake of 2-dGlc (*n* = 6). The zero-trans uptake of 2-dGlc with RU 38486 and dexamethasone was not significantly different from the control (*n* = 6). In all conditions, preloading with 3-OMG (40 mM) (infinite-trans exchange uptake) significantly increased the uptake of 2-d[2,6-³H]Glc (0.1 mM) when compared with zero-trans uptake (*P* < 0.001, in all cases; *n* = 6). † *P* < 0.01; ^{n.s.}, not significant (test versus control zero-trans uptake); *** *P* < 0.001 (test versus zero-trans uptake).

Type of uptake	2-dGlc uptake (μmol · 5 min ⁻¹ · ml of cell water ⁻¹)			
	Control	Dexa (0.1 μM)	RU 38486 (1 μM)	Dexa + RU 38486
zero-trans	0.43 ± 0.01	0.34 ± 0.02†	0.44 ± 0.01 ^{n.s.}	0.44 ± 0.01 ^{n.s.}
infinite-trans	0.57 ± 0.01***	0.58 ± 0.01***	0.60 ± 0.03***	0.61 ± 0.02***
<i>n</i> ...	6	6	6	6

Fig. 6. Dexamethasone (0–10 μM) and RU 38486 (1 μM) effects on PMA-stimulated HMPS activity

Macrophages were incubated for 4 h at 37 °C with various concentrations of dexamethasone (0–10 μM) and in the presence or absence of RU 38486 (1 μM). The cells were then washed and the uptake of 2-d[1-³H]Glc and 2-d[2,6-³H]Glc (0.1 mM) over 15 min with PMA (40 nM) was measured in parallel experiments. HMPS activity (μmol · 15 min⁻¹ · 3 × 10⁹ cells⁻¹) was calculated from the difference in uptake of the labels and expressed as a percentage of the value in the absence of dexamethasone. All concentrations of dexamethasone significantly decreased PMA-induced HMPS activity (*P* < 0.01, in all cases; *n* = 6) and the effect was concentration-dependent. At 0.1 and 1, but not 10 μM-dexamethasone, RU 38486 (1 μM) significantly increased the HMPS activity compared with dexamethasone alone (*P* < 0.01, in both cases; *n* = 6). □, Control; ■, RU 38486 (1 μM).

without RU 38486 (1 μM). The activity of the HMPS was then determined by measuring the uptake of 2-d[1-³H]Glc and 2-d[2,6-³H]Glc (0.1 mM) for 15 min in parallel experiments in the presence of PMA (40 nM), a potent activator of the HMPS in rat peritoneal macrophages (Rist *et al.*, 1990). The difference between the uptake of the two labels of 2-dGlc in μmol · 15 min⁻¹ · 3 × 10⁹ cells⁻¹ is equivalent to the amount of sugar metabolized by the HMPS pathway.

Fig. 6. shows a plot of the data with HMPS activity expressed as a percentage of the control value, i.e. in the absence of dexamethasone. Dexamethasone significantly decreased the PMA-induced HMPS activity at all concentrations of the glucocorticoid (*P* < 0.01, in all cases; *n* = 6), with uptake of 0.1 mM-2-dGlc inhibited by 60 % at 10 μM-dexamethasone. The presence of RU 38486 (1 μM) with dexamethasone reversed the inhibitory effects of the glucocorticoid on PMA-induced HMPS activity. At 0.1 and 1 μM-dexamethasone, RU 38486 (1 μM) significantly increased HMPS activity above that with dexamethasone alone (*P* < 0.01, in both cases; *n* = 6). However,

Fig. 7. Comparison of the effects of dexamethasone and cytochalasin B on 2-dGlc uptake and HMPS activity

Macrophages were incubated for 4 h at 37 °C with various concentrations of dexamethasone (0–10 μM), and then the PMA-stimulated (40 nM) uptake of 2-d[1-³H]Glc and 2-d[2,6-³H]Glc (0.1 mM) over 15 min was measured. In parallel experiments, the uptake of the two labelled sugars was followed over 15 min, but in the presence of PMA (40 nM) and various concentrations of cytochalasin B (0–1 μM). Uptake of 2-d[2,6-³H]Glc and HMPS activity were calculated and expressed as a percentage of the control value in the absence of inhibitor. The ratio of percentage HMPS activity to percentage 2-d[2,6-³H]Glc uptake was plotted against percentage inhibition of 2-d[2,6-³H]Glc uptake and lines fitted by linear least-squares regression. The gradient of the line fitted to the dexamethasone (■) data was -0.009 ± 0.003 (*n* = 3), and was significantly different from the gradient of the line for cytochalasin B (□) (0.0036 ± 0.0005, *n* = 5; *P* < 0.01).

RU 38486 (1 μM) did not significantly increase HMPS activity when the concentration of dexamethasone was raised to 10 μM.

Comparison of the effects of various concentrations of dexamethasone and cytochalasin B on 2-d[2,6-³H]Glc uptake and HMPS activity

Macrophages were preincubated for 4 h at 37 °C with or without various concentrations of dexamethasone (0–10 μM), and then the PMA-dependent (40 nM) uptake of 2-d[1-³H]Glc and 2-d[2,6-³H]Glc (0.1 mM) over 15 min was measured in the presence or absence of the sugar-transport inhibitor cytochalasin B (0–1 μM). From these results the uptake of 2-d[2,6-³H]Glc and the HMPS activity were calculated as a percentage of the value in the absence of inhibitor, and then the ratio of percentage HMPS activity to percentage 2-d[2,6-³H]Glc uptake was plotted against percentage inhibition of 2-d[2,6-³H]Glc uptake (Fig. 7). Lines were fitted to the data by linear least-squares regression.

In the presence of dexamethasone there is a decrease in the ratio between HMPS activity and 2-d[2,6-³H]Glc uptake with

increasing 2-d[2,6-³H]Glc inhibition. This indicates that there is differential inhibition of the HMPS and 2-d[2,6-³H]Glc uptake with dexamethasone treatment. In contrast, when macrophages are incubated with various concentrations of cytochalasin B, there is a small, but not significant, increase in the ratio of HMPS activity to 2-d[2,6-³H]Glc uptake with increasing 2-d[2,6-³H]Glc inhibition. This finding suggests that both 2-d[2,6-³H]Glc uptake and HMPS activity are inhibited to the same extent by increasing concentrations of cytochalasin B. The gradient of the line fitted to the dexamethasone data was -0.009 ± 0.003 ($n = 3$), and this was significantly different from the gradient for cytochalasin B (0.0036 ± 0.0005 , $n = 5$; $P < 0.01$).

The data indicate that dexamethasone and cytochalasin B inhibit HMPS activity and 2-d[2,6-³H]Glc uptake in PMA-activated rat macrophages in different ways. Cytochalasin B has been shown to inhibit HMPS activity and 2-d[2,6-³H]Glc uptake equally with increasing inhibition of 2-d[2,6-³H]Glc uptake, and this suggests a single site of action of this inhibitor. It is thought that cytochalasin B inhibits sugar transport in human erythrocytes by binding to the endofacial surface of the glucose transporter (Basketter & Widdas, 1978; Devés & Krupka, 1978), and this correlates with the results presented here, since binding of inhibitor to the sugar transporter will inhibit both 2-d[2,6-³H]Glc uptake and HMPS activity.

Dexamethasone is shown to differentially inhibit HMPS activity and 2-d[2,6-³H]Glc uptake with increasing inhibition of 2-d[2,6-³H]Glc uptake (Fig. 7). This finding indicates that dexamethasone may inhibit sugar uptake and HMPS activity, not only by decreasing the coupling between hexokinase and the sugar transporter (as reported above), but also at one or more additional points between the transporter and the HMPS pathway.

DISCUSSION

The results indicate, as previously reported for rat thymocytes (Naftalin & Rist, 1990), that dexamethasone decreases 2-dGlc uptake and accumulation in rat peritoneal macrophages by

reducing the activity of hexokinase at the endofacial surface of the glucose transporter. The evidence for this is as follows: (1) dexamethasone significantly increased the K_m for *zero-trans* uptake of 2-dGlc, without significantly altering the V_{max} (Fig. 4.); (2) the rate of exit of 2-dGlc from cells preloaded with sugar was significantly increased by dexamethasone (Fig. 5); (3) the free-sugar accumulation within the cytosol of the cells was significantly decreased by dexamethasone (Table 1). Furthermore, the absence of any effect of dexamethasone on the maximal rate of exchange uptake of 2-dGlc into cells loaded with 3-OMG (Table 2) indicates that dexamethasone reduces hexokinase trapping of 2-dGlc rather than the number of transporters present within the membrane.

It has been reported that dexamethasone inhibits glucose transport in rat adipocytes by decreasing the number of transporters in the plasma membrane (Carter-Su & Okamoto, 1985). However, an alternative model has been proposed which can explain the inhibitory effects of glucocorticoids on sugar transport (Naftalin & Smith, 1987). This model suggests a 'loose coupling' between sugar transport and hexokinase activity and envisages that sugar emerging at the endofacial surface of the transporter can either be released into the cytosol, or remain on the transporter and be returned to the exofacial surface in a 'futile cycle', or be phosphorylated by hexokinase at the endofacial surface and then released into the cytosol. The hexokinase reaction forms impermeant sugar phosphate, thus reducing the recycling of permeant sugar via futile cycles and so increasing the net rate of uptake of sugar. It follows that a glucocorticoid-dependent reduction in hexokinase activity at the endofacial surface of the transporter would lead to a number of predictions. Firstly, there would be an increase in the K_m for *zero-trans* uptake of sugar, due to increased binding of free sugar at the endofacial surface of the transporter. Secondly, the rate of exit of free sugar would increase, because reduced phosphorylation of cytosolic free sugar bound to the endofacial surface of the transporter would allow the sugar to escape from the cell. Thirdly, there would be decreased accumulation of free sugar within the cell compared with the control, due to the combination of the first two effects together with the action of sugar phosphatases.

The finding that the results reported here agree with all the

Scheme 1. Interactions between the transport of sugar, HMPS activity and superoxide production and the possible sites of action of PMA, dexamethasone and cytochalasin B in rat peritoneal macrophages

predictions of the 'loose coupling' model, indicate that there is a dexamethasone-dependent decrease in the coupling of hexokinase activity and sugar transport in rat peritoneal macrophages.

Prolonged incubation in the presence of glucocorticosteroids leads to lymphocytolysis, and it has been suggested that this is due to induction of nucleolytic products via a receptor-mediated pathway (Compton & Cidlowski, 1987). However, recent work indicates that nucleosomal DNA cleavage is due to activation of an endogenous endonuclease(s) and does not require expression of a new nuclease (Alnemri & Litwack, 1989).

RU 38486 shows powerful anti-glucocorticoid activity *in vivo* and *in vitro* and is also a potent anti-progesterone (Philibert, 1984). It has been suggested that this antagonist effect is related to strong binding to the cytosolic glucocorticoid receptor, forming a stable complex (Moguilewsky & Philibert, 1984). The relative binding affinity of RU 38486 is three times higher than that of dexamethasone at 0 °C in the rat thymus, and this agrees with our finding that the K_i for RU 38486 on the dexamethasone-dependent inhibition of 2-d[2,6-³H]Glc uptake in rat macrophages ($0.04 \pm 0.01 \mu\text{M}$) is lower than the K_i for dexamethasone itself ($0.10 \pm 0.06 \mu\text{M}$). It has been reported that RU 38486 has no agonist effects (Philibert, 1984), but our results show that RU 38486 treatment leads to a small but significant increase in 2-dGlc uptake in some cases, but not others (Fig. 2, but not Table 2). This may be due to the presence of inhibitory levels of endogenous glucocorticoids in some cell preparations which are blocked by RU 38486, thus increasing sugar uptake.

These results indicate that dexamethasone induces a glucocorticoid-receptor-mediated response which leads to a loosening between hexokinase activity and glucose-transport activity and also uncoupling of the HMPS with hexokinase. This suggests a dual site of dexamethasone-induced inhibition of superoxide production and a schematic representation of the interactions between dexamethasone, PMA and cytochalasin B, and the various metabolic processes in the rat macrophage are shown in Scheme 1. Also, the inhibitory effect of dexamethasone on sugar transport and HMPS activity can be overcome when the external sugar concentration is increased, indicating that the anti-inflammatory effect of glucocorticoids in reducing superoxide production may only occur in macrophages in conditions where glucose uptake is low, e.g. in an inflamed hypoxic joint.

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