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The relationship between sugar metabolism, transport and superoxide radical production in rat peritoneal macrophages

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Dexamethasone inhibits sugar-dependent phorbol myristate acetate (PMA)-stimulated superoxide production and 2-deoxy-D-glucose (2-dGlc) transport in rat peritoneal macrophages (Rist, R.J., Jones, G.E. and Naftalin, R.J. (1991) Biochem. J. 278, 119–128; Rist, R.J. and Naftalin, R.J. (1991) Biochem J. 278, 129–135). Here it is shown that with glucose as a substrate, dexamethasone (0.1 μM) acts as a non-competitive inhibitor of PMA-induced superoxide production; decreasing the maximal rate of superoxide production ($P < 0.001$) without altering the K_m. In contrast, with 2-dGlc as a substrate, dexamethasone shows competitive inhibition of PMA-stimulated superoxide production; increasing the K_m of superoxide production, ($P < 0.001$) without altering the V_{max}. The maximal rate of PMA-stimulated superoxide production with glucose as substrate is 10–12-fold in excess of the maximal rate with 2-dGlc as substrate. Diphenylene iodonium (DPI) is a non-competitive inhibitor of PMA-stimulated glucose-dependent superoxide production in macrophages, ($K_i = 1–5$ μM) and significantly reduces the activity of the PMA-induced hexose monophosphate shunt, (HMPS) ($P < 0.01$). However, DPI (1 μM) has no significant effect on the PMA-induced increase in 2-dGlc uptake, suggesting that the stimulus for HMPS activity and superoxide production is separate from the stimulus for hexose transport. A model is described which explains the observed differences in hexose transport and glucose- and 2-dGlc-dependent superoxide production in terms of the differences in metabolism of the two sugars. Accumulation of free 2-dGlc within the cytosol leads to saturation of hexokinase and hence, the effects of PMA and dexamethasone, which alter the coupling between hexokinase and the transporter, are only observed at low concentrations of 2-dGlc, where it is accumulated to sub-saturating levels. Since glucose is completely metabolized within the cell, PMA and dexamethasone increase and decrease, respectively, net uptake of sugar and superoxide production at all glucose concentrations.

Introduction

Macrophages are phagocytic cells of the immune system, which have a vital role in killing infecting micro-organisms [3]. Activation of these cells by a variety of chemotactic and phagocytic agents results in generation of superoxide anion radicals (O_2^-), from oxygen via a membrane-bound complex containing NADPH oxidase [4]. This activation is characterized by an increase of up to 50-fold in O_2 consumption, the 'respiratory burst', involving the partial reduction of oxygen via a flavoprotein and a *b*-type cytochrome, to produce superoxide at the expense of NADPH [5], which is in turn produced from glucose-6-phosphate (Glc-6-*P*) by the hexose monophosphate shunt (HMPS) [6].

In rat peritoneal macrophages, superoxide generation is totally dependent on the uptake of extracellular hexose and it has been suggested that the respiratory burst results from enhanced coupling of the sugar transport process to hexokinase activity at the endofacial surface of the glucose transporter [1]. Coupling between hexokinase activity and sugar transport confers a kinetic advantage, as it reduces the number of 'futile' transport cycles by retarding backflux of free sugar via the carrier, thus increasing net influx. Direct evidence for this view has recently been obtained by localisation studies of hexokinase within peritoneal macrophages. PMA, a potent activator of the respiratory burst in macrophages, stimulates translocation of hexokinase to the cell cortex. The density of hexokinase at the cell membrane is increased by 76% above that found in inactive cells. The translocation process is apparently mediated by cytoskeletal structures [7].

The detailed kinetic evidence for coupling of hexokinase to transport is derived from the asymmetric transport of the partially-metabolised sugar 2-dGlc by macrophages [1,2,8]. Influx of 2-dGlc is enhanced by PMA, whilst exit of free sugar is retarded. 2-dGlc-6-*P* produced at the endofacial surface of the cell mem-

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brane by bound hexokinase is released into the cytosol and is recycled to give free 2-dGlc by the action of phosphatases. The free sugar in the cytosol is prevented from leaving the cell by hexokinase action at the plasma membrane, thus both free and phosphorylated sugar can accumulate within the cytosol [9].

Dexamethasone is a synthetic glucocorticoid which has been shown to inhibit superoxide production in macrophages [10,11]; an observation that may at least partly explain the dexamethasone-dependent reduction in macrophage tumoricidal and anti-microbial activity [12,13]. Dexamethasone also inhibits macrophage sugar transport [14] and it has been reported that this is due to a reduced coupling between transport and hexokinase activity which leads to a marked reduction in transport asymmetry, i.e., sugar uptake is reduced, free sugar exit increased and free sugar accumulation is reduced [2]. At low external concentrations of 2-dGlc, dexamethasone also reduces hexose monophosphate shunt metabolism and inhibition studies suggest multiple sites of inhibition by the steroid [2].

Glucose and 2-dGlc are transported via the glucose transporter at similar rates in isolated adipocytes [15] and both have the same maximal rate of phosphorylation by hexokinase in rat brain [16]. However, it has been shown in human red cells that the rate of utilization of 2-dGlc-6-*P* by glucose 6-phosphate dehydrogenase is less than 5% of the rate of utilization of Glc-6-*P* [17]. As a result, 2-dGlc is metabolised slowly via the HMPS and, as we demonstrate here, this allows only a low rate of superoxide production in PMA-stimulated cells.

The rapid metabolism of glucose, both via the HMPS and the glycolytic pathway, makes glucose transport into macrophages difficult to monitor, except indirectly using the glucose- or 2-dGlc-dependent superoxide production in PMA-stimulated cells. However, even with this limitation, it is possible to demonstrate major differences in the ways that glucose and 2-dGlc are processed during the respiratory burst and from this useful insights into the mechanism of sugar processing in the macrophage can be obtained.

Our measurements of 2-dGlc transport show that PMA stimulates 2-dGlc uptake only in the concentration range 0–2 mM, but not at higher concentrations [1]. Similarly, dexamethasone-dependent inhibition of 2-dGlc uptake is only observed at low sugar concentrations, i.e., PMA and dexamethasone alter transport of 2-dGlc by lowering or raising the apparent K_m of 2-dGlc uptake, but the V_{max} values are unaffected [2]. This means that when 2-dGlc is present at raised concentrations no significant effects of these modulators are observed.

These data present us with a paradox, PMA has been reported to increase superoxide production [18,19] and dexamethasone to reduce stimulated superoxide production [11,19] in phagocytes with concentrations of D-glucose present in the physiological range 5–10 mM, hence they affect the V_{max} rather than the K_m of sugar transport and metabolism.

In this paper the differences between superoxide production in macrophages with either 2-dGlc or glucose as substrates are investigated and it will be shown that the kinetic differences are a consequence of the low rate of metabolism of 2-dGlc-6-*P* via the HMPS and its failure to be metabolised via the glycolytic pathway.

Materials and Methods

Cell preparation

Rat peritoneal macrophages were prepared as described previously [8]. Briefly, Wistar rats (150–200 g body wt.) were anaesthetised with diethyl ether and then killed by cervical dislocation. Immediately, a 10-ml sample of Hepes-buffered saline (Hepes (*N*-2-hydroxyethylpiperizine-*N'*-2-ethanesulfonic acid), 5 mM; NaCl, 140 mM; KCl, 5 mM; $MgCl_2$, 1.2 mM) (pH 7.3) at 37°C, was injected into the peritoneal cavity of the rats. After 5–10 min gentle massage of the abdomen, the cell suspension was withdrawn and centrifuged ($300 \times g$ for 3 min), before being washed twice with saline. Each experiment required three or four rats, with each animal yielding approx. $(1-2) \cdot 10^7$ macrophages.

Microscopy and cell counting

The preparation described did not result in death or damage to the cells. There was little or no debris detectable with light microscopy and 95% of cells excluded Evans' Blue stain. Differential counts of stained preparations of the peritoneal washings showed > 90% macrophages and monocytes.

The mean cell volume of the macrophages was estimated by measurement of the 3-*O*-methyl-D-glucose space and by using an Elzone 280PC particle analyser (Particle Data). The estimate obtained for macrophage volume was 330 fl and thus $3 \cdot 10^9$ cells are about 1 ml cell water. All sugar uptake rates etc. are normalized to $3 \cdot 10^9$ cells. Cell numbers were analysed using the Elzone 280PC with macrophage suspensions diluted to between $5 \cdot 10^5$ and $1 \cdot 10^6$ cells/ml.

Transport methods

Net uptake. Sugar uptake into macrophages was measured using 2-deoxy-D-[1-^{3}H]glucose (Amersham UK, initial specific activity 17 Ci/mmol; final concentration 0.5 μCi/ml), or 2-deoxy-D-[2,6-^{3}H]glucose (Amersham UK, initial specific activity 42.5 Ci/mmol; final concentration 0.5 μCi/ml). After incubation, sugar uptakes were halted by adding iso-osmotic ice-cold stopping solution, consisting of saline containing phloretin and $HgCl_2$ at final concentrations of 100 μM and 1 μM, respectively. The passive uptake of sugar

was determined by measuring uptake in the presence of stopping solution and all uptake rates are expressed as phloretin-sensitive uptakes.

After adding stopping solution, the macrophages were centrifuged for 3 min at $3000 \times g$, the supernatants removed and a sample retained for the determination of the extracellular radioactivity. The cells were then washed twice by suspension and re-centrifugation in fresh ice-cold stopping solution to remove extracellular isotope. Finally, the cell pellets were lysed by vortexing in distilled water, and 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 toluene (BDH), 1 litre Synperonic NX (Durham Chemicals Distributors) and 5 g of 2,5-diphenyloxazole (Sigma).

Hexose monophosphate shunt. HMPS activity of macrophages was measured using the 'double-label' method previously reported [8], with cells being incubated in 2-deoxy-D-[1-^{3}H]Glc or 2-deoxy-D-[2,6-^{3}H]Glc. In the HMPS, the 1-position carbon atom is cleaved from the hexose. When cells with an active HMPS are incubated with 2-deoxy-D-[1-^{3}H]Glc, the label is lost as [^{3}H]H_2O, but label at other positions is retained within the cytosol. It follows that the difference between the net uptake of 2-deoxy-D-[1-^{3}H]Glc and 2-deoxy-D-[2,6-^{3}H]Glc indicates the activity of the HMPS pathway, whereas the rate of uptake of 2-deoxy-D-[2,6-^{3}H]Glc measures the true rate of sugar uptake via the plasma membrane sugar transport system and the accumulation of unphosphorylated 2-deoxy-D-[1-^{3}H]Glc within the cytosol measures the level of free 2-dGlc unaltered by HMPS activity.

Estimation of phosphorylated and non-phosphorylated sugar

A Whatman DE81 anion-exchange filter was washed with 2 ml ice-cold distilled water by suction via a Buchner filtration tube and the water discarded; then 1 ml of cell lysate (at 4°C) was filtered and the filtrate retained. Next, the filter was washed with 2 ml of cold distilled water and this second filtrate added to the first. The combined filtrate was sampled for free sugar and the remainder discarded. The sugar phosphate retained on the filter was removed by washing with 2 ml 0.5 M HCl, and a sample was counted. Total, free sugar and hexose phosphates per $3 \cdot 10^9$ macrophages were estimated after correction for quench and dilution. This method of separation was previously shown to give accurate results [20].

Measurement of superoxide production

Superoxide anion production by macrophages was measured as described previously [21]. Cytochrome *c* (20 μM) (horse heart; Sigma) was present in the Hepes buffer during incubation of the cells under the various treatments and the reaction was stopped by placing a 1.5-ml sample of the suspension in an Eppendorf tube on ice. Following centrifugation at $3000 \times g$ for 3 min at 4°C, the supernatant was removed and stored on ice. The absorbance spectrum of the individual samples was then measured over the range 540 to 560 nm in a Cecil CE 2020 spectrophotometer.

Reduced cytochrome *c* in the sample, and thus superoxide generated, was calculated using an absorbance coefficient of 21.1 mM^{-1} cm^{-1} [22] and expressed as $\mu mol/3 \cdot 10^9$ cells per h. Each condition had an identical control containing 10 μg/ml superoxide dismutase (SOD) and every value is expressed as the SOD-inhibitable superoxide production.

Modelling with Stella II

Simulation. Stella II is a computer-modelling package for the Macintosh computer which uses the computer's graphical interface to set up the simulation of differential equations. It offers many practical advantages in terms of speed of model construction and sophisticated use of the computer graphing facilities to produce numerical solutions with fourth-order Runge Kutta numerical integration procedures.

Model. The model used here is an adaptation of a previous model [9]. It consists of a simple facilitated transport system for sugars such as has been described for rat red cells; namely a symmetrical transport system of the Glut-1 type. On the endofacial surface of the transporter there is membrane-bound hexokinase which can metabolise the sugar bound to the transporter to hexose 6-phosphate prior to equilibration with the cytosolic pool. A proportion of the hexose can diffuse directly into the cytosol without alteration. Once in the cytosolic pool, the hexose can be transformed to hexose 6-phosphate by cytosolic hexokinase. Hexose 6-phosphate bound to the endofacial surface also equilibrates with the cytosolic pool of hexose 6-phosphate; endofacial hexose 6-phosphate can be metabolised by glucose-6-phosphate dehydrogenase via the HMPS to produce superoxide and pentose phosphates. Hexose 6-phosphate in the cytosol is subject to degradation by phosphatases to regenerate glucose and can be utilised by the glycolytic pathway to generate lactate.

The major differences between 2-dGlc and glucose are (a) that metabolism of 2-dGlc via the HMPS shunt is much slower than for glucose because 2-dGlc is a poor substrate for glucose-6-phosphate dehydrogenase and (b) there is no metabolism of 2-dGlc-6-*P* within the cytosol via the glycolytic pathway, although it is subject to phosphorolysis by phosphatases within the cytosol. The rates of transport across the cell membrane; metabolism via hexokinase at the endofacial surface and within the cytosol of the two sugars are identical. It will be shown that these differences are all that is required to explain the differences in the effects

of dexamethasone and PMA on both 2-dGlc- and glucose-dependent transport and superoxide production by macrophages. The modelling process involves simulation of the following transport and metabolic transformations:

(1) Facilitated diffusion of hexose across the membrane into the cortical endofacial cell compartment: (net flow is taken as the difference between the saturation levels of transporter at the exo and endofacial surfaces, the affinity of the transporter is assumed to be symmetrical and equal to 1 mM for both glucose and 2-dGlc; the V_{max} of the transport system is also assumed to be symmetrical and that for glucose and 2-dGlc equal: it was unnecessary to simulate exchange transport, as this simulation is concerned solely with net fluxes).

(2) Sub-endofacial hexokinase activity converts either glucose, or 2-dGlc to glucose/2-dGlc-6-*P*. (K_m and V_{max} are the same for both glucose and 2-dGlc, but the V_{max} can be controlled either to simulate activation (PMA) or inhibition (dexamethasone)).

(3) HMPS activation by PMA metabolises glucose/2-dGlc-6-*P* to pentose phosphate and generates superoxide radicals. (The V_{max} of this reaction can be varied to simulate either the difference between 2-dGlc and glucose, or the effect of DPI).

(4) Diffusion of glucose/2-dGlc and Glc-6-*P*/2-dGlc-6-*P* between the cytosolic core and cell cortex. (The rates of both hexoses and hexose phosphates are assumed to be the same).

(5) Simulation of effects of sugars (2-dGlc or glucose) at varying concentrations in the presence of inhibitors and activators of transport, hexokinase coupling to the membrane and superoxide radical production.

(6) Glycolysis (irreversible loss of glucose-6-*P* but not 2-dGlc-6-*P* from the cytosolic pool).

(7) Phosphorolysis of cytosolic glucose-6-*P* and 2-dGlc-6-*P* to free glucose (the K_m and V_{max} of this reaction are assumed to be similar for both hexose phosphates).

(8) Cytosolic hexokinase (cytosolic free glucose and free 2-dGlc are converted to glucose-6-*P* or 2-dGlc-6-*P* with the same K_m and V_{max}).

Fig. 1 is a diagrammatic representation of the model system used, showing the relationships between the various metabolic processes and their Michaelis-Menten parameters, i.e., membrane transport, hexokinase activity, glycolysis, etc., and the compartments of the cell.

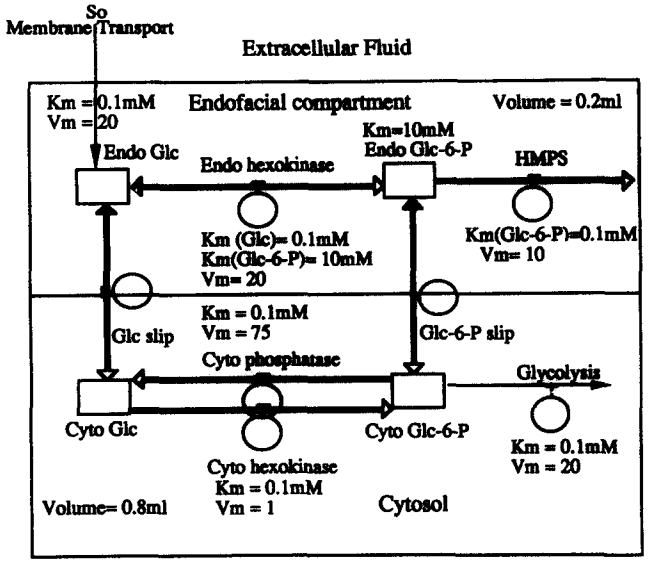

Fig. 1. Diagrammatic representation of the model system.

Results

Effects of variation of hexose concentration on PMA-induced superoxide production

The effects of altering the hexose substrate concentration on PMA-induced superoxide production in rat peritoneal macrophages are shown in Fig. 2. With both glucose and 2-dGlc as substrates, there is a concentration-dependent increase in superoxide production, which is sensitive to superoxide dismutase (SOD). The data have been plotted using the Kaleidagraph non-linear least squares program (Marquard algorithm) to fit the Michaelis-Menten equation. The K_m values for glucose and 2-dGlc are 0.28 ± 0.08 and 0.09 ± 0.01 mM, respectively; the V_{max} values are 67.03 ± 3.69 and 5.77 ± 0.16 μmol/20 min per ml cell water. Thus, both the V_{max} and K_m for 2-dGlc are significantly lower than the corresponding values for glucose ($P < 0.001$).

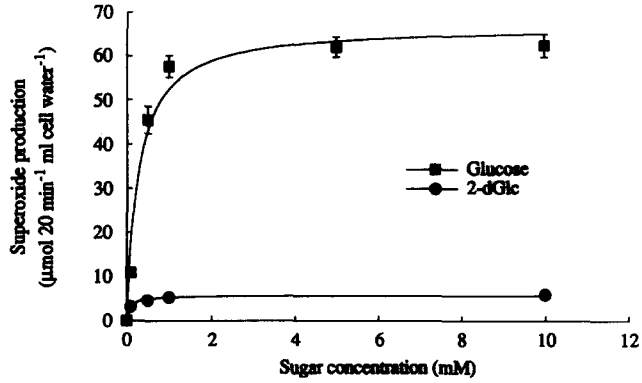

Fig. 2. Effects of variation of hexose concentration on PMA-dependent superoxide production. Peritoneal macrophages were pre-incubated for 4 h in sugar-free Hepes-buffered saline at 37°C, then washed and transferred to buffer containing varying concentrations of either glucose or 2-dGlc (0–10 mM), cytochrome *c* (20 μM) and PMA (40 nM). The production of superoxide was then measured over the next 20 min at 37°C (see Materials and Methods) and the results (means ± S.E.) are expressed as SOD-inhibitable superoxide production with zero-sugar value subtracted (μmol/20 min per ml cell water). The data are plotted by a non-linear least-squares fit to the Michaelis-Menten equation (KaleidaGraph 2.1, Abelbeck Software). Both the V_{max} and K_m for 2-dGlc (●) are significantly lower than the corresponding values for glucose (■); $P < 0.001$.

The maximal rate of superoxide production in the presence of glucose (67.03 ± 3.69 μmol/20 min per ml cell water) is 10–12-fold in excess of the maximal rate of superoxide with 2-dGlc present (5.77 ± 0.16 μmol/20 min per ml cell water). Since both sugars are transported via the same membrane transporter, as can be deduced from the fact that cytochalasin B inhibits superoxide production completely [1], and the V_{max} for 2-dGlc transport is similar to that of glucose via the erythrocyte transporter [23], this result indicates that the low rate of 2-dGlc-dependent superoxide production in rat peritoneal macrophages is rate-limited by its low rate of metabolism.

Identical experiments to those described above, but using mannose and fructose as substrates for PMA-induced superoxide production were performed (data not shown). The maximal rates of superoxide production with these hexoses were found to be between the values obtained for glucose and 2-dGlc (mannose, 54.85 ± 3.09 μmol/20 min per ml cell water; fructose, 11.97 ± 1.05 μmol/20 min per ml cell water; $P < 0.001$), whilst the K_m values were not significantly different from that of glucose (K_m mannose, 0.38 ± 0.08 mM; K_m fructose, 0.39 ± 0.13 mM). These results indicate that fructose transport, but not mannose transport may rate limit superoxide production in rat macrophages.

Effects of dexamethasone on glucose-dependent superoxide production

The effects of varying concentrations of dexamethasone on PMA-stimulated glucose-dependent superoxide production in peritoneal macrophages are shown in Fig. 3a. There is a significant dexamethasone-dependent decrease in the maximal rate of superoxide production induced by PMA (control $V_{max} = 61.02 \pm 4.22$ μmol/20 min per ml cell water; dexamethasone (0.01 μM) $V_{max} = 46.45 \pm 3.09$ μmol/20 min per ml cell water; dexamethasone (0.1 μM) $V_{max} = 27.28 \pm 1.14$ μmol/20 min per ml cell water; $P < 0.001$, in both cases), but no significant change in the K_m of this process (control $K_m = 0.28 \pm 0.08$ mM; dexamethasone (0.01 μM) $K_m = 0.32 \pm 0.08$ mM; dexamethasone (0.1 μM) $K_m = 0.35 \pm 0.06$ mM), indicating that dexamethasone is a non-competitive inhibitor of glucose-dependent superoxide production. The K_i for dexamethasone can be calculated from the relationship, $K_i = [\text{inhibitor}]/((K_p/K_m) - 1)$, where K_p is the K_m in the presence of the inhibitor. The data give a K_i of 0.07 ± 0.02 μM for 0.01 μM dexamethasone and a K_i of 0.40 ± 0.10 μM for 0.1 μM dexamethasone. This result contrasts with the apparent competitive inhibition by dexamethasone of PMA-stimulated 2-dGlc uptake reported previously [2].

Reversal by RU 38486 of dexamethasone inhibition of glucose-dependent superoxide production

The specificity of the inhibition by dexamethasone of the PMA-stimulated glucose-dependent superoxide production is tested by observing the extent of its reversibility by simultaneous exposure to the competitive inhibitor of glucocorticoid action, RU38486 [24,25]. The non-competitive inhibition by dexamethasone (0.01 μM) of the maximal rate of glucose-dependent super-

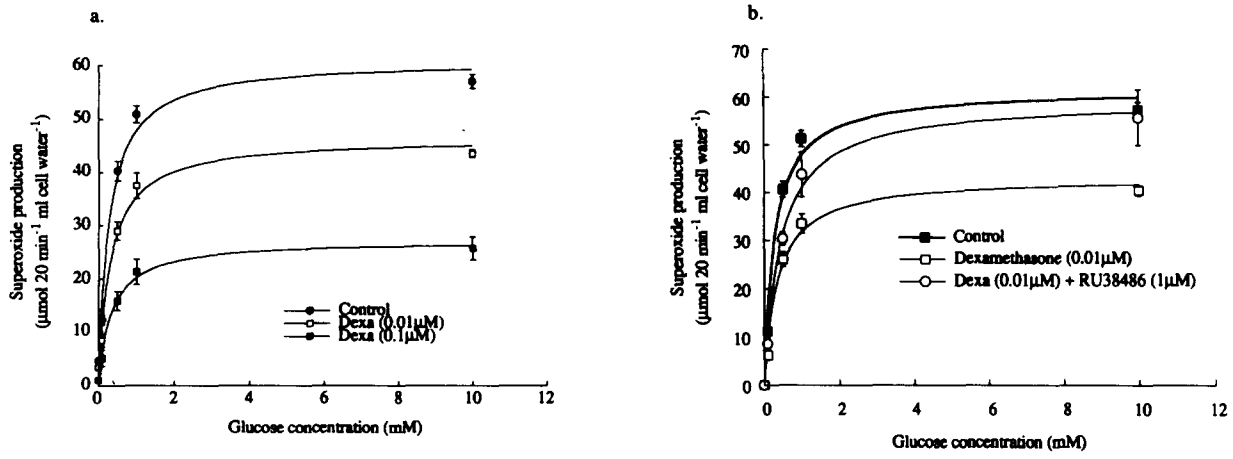

Fig. 3. Effects of dexamethasone on glucose-dependent superoxide production. (a) Macrophages were pre-incubated with or without dexamethasone (0.01 or 0.1 μM) for 4 h in sugar-free Hepes-buffered saline at 37°C, then washed and transferred to buffer containing varying concentrations of glucose (0–10 mM), cytochrome *c* (20 μM) and PMA (40 nM). The production of superoxide over the next 20 min was calculated from the reduction of the cytochrome *c* (see Materials and Methods) and the results, (means ± S.E.) are expressed as SOD-inhibitable superoxide production (μmol/20 min per ml cell water). The data are plotted using KaleidaGraph 2.1 (see legend to Fig. 2) and show that dexamethasone significantly decreases the maximal rate of superoxide production induced by PMA ($P < 0.001$, in both cases), but there is no significant change in the K_m. Control, (●); dexamethasone (0.01 μM), (□); dexamethasone (0.1 μM), (■). (b) Macrophages were pre-incubated with or without dexamethasone (0.01 μM) and RU 38486 (1 μM) for 4 h and then treated as described in the legend for Fig. 3a. Dexamethasone (0.01 μM) significantly reduced the maximal rate of PMA-induced glucose-dependent superoxide, ($P < 0.001$), and RU 38486 (1 μM) reversed the inhibitory effects of dexamethasone. The K_m values for control (■), dexamethasone (□) and dexamethasone + RU38486 (○) were not significantly different.

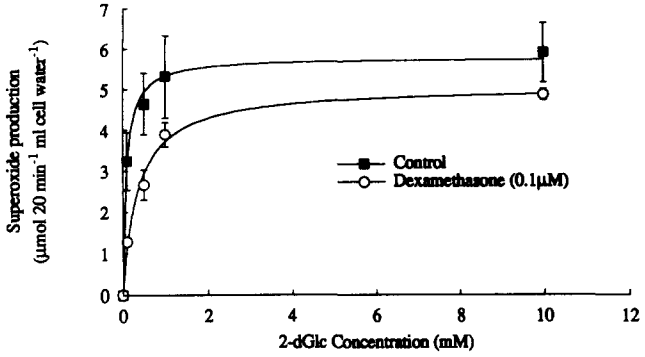

Fig. 4. Effects of dexamethasone on 2-dGlc-dependent superoxide production. Macrophages were pre-incubated with or without dexamethasone (0.1 μM) for 4 h at 37°C in sugar-free medium and then transferred to varying concentrations of 2-dGlc with PMA (40 nM) and cytochrome *c* (20 μM). Superoxide production over 20 min was determined and the data processed as described in the legend to Fig. 2. Dexamethasone significantly increased the K_m of PMA-stimulated 2-dGlc-dependent superoxide production ($P < 0.001$), without significantly altering the maximal rate of production. Control, (■); dexamethasone (0.1 μM), (○).

oxide production is fully reversed by RU38486 (1 μM; Fig. 3b). The maximal rates of PMA-induced superoxide production were: control, 61.38 ± 3.92; dexamethasone (0.01 μM), 42.96 ± 2.45; dexamethasone + RU38486 (1 μM), 59.12 ± 2.51 μmol/20 min per ml cell water.

Effects of dexamethasone on 2-dGlc-dependent superoxide production

The effect of 4 h exposure to dexamethasone (0.1 μM) on PMA-induced 2-dGlc-dependent superoxide production is shown in Fig. 4. Dexamethasone acts as a competitive inhibitor of superoxide production; at low concentrations of 2-dGlc there is significant inhibition of superoxide production, whereas at high concentrations (5–10 mM) inhibition is negligible. These data are consistent with the competitive inhibition of 2-dGlc influx by dexamethasone shown previously [2]. The apparent K_m changes significantly from a control value of 0.09 ± 0.01 mM to 0.36 ± 0.06 mM with dexamethasone (0.1 μM) present ($P < 0.001$). The V_{max} in the control cells is 5.77 ± 0.16 μmol/20 min per ml cell water and is not significantly different from that found in the presence of dexamethasone (V_{max} = 5.05 ± 0.23 μmol/20 min per ml cell water).

These data contrast with those shown in Fig. 3a with glucose as a substrate; where it was shown that dexamethasone acts as a non-competitive inhibitor of superoxide production. The maximal rate of superoxide production is 10–12-fold higher with glucose than with 2-dGlc as a substrate.

Effects of dexamethasone on 2-dGlc accumulation in control and PMA-treated cells

The effects of a 90-min incubation with PMA (40 nM) and dexamethasone (0.1 μM) separately and together on 2-dGlc (0.1 mM) accumulation are shown in Table I. PMA significantly increases steady-state accumulation of total and free sugar and 2-dGlc-6-*P* above control values ($P < 0.001$ for total and free sugar; $P < 0.05$ for 2-dGlc-6-*P*). Dexamethasone (0.1 μM) significantly decreases steady-state accumulation of total sugar in both control and PMA-treated cells ($P < 0.001$ for dexamethasone vs. control; $P < 0.01$ for PMA + dexamethasone vs. PMA alone).

It has been shown previously that dexamethasone competitively inhibits the PMA-stimulated 2-dGlc uptake [2]. These findings indicate that at low external concentrations of 2-dGlc the dexamethasone-dependent decrease in membrane-bound hexokinase activity is sufficient to reduce sugar transport, but the inhibition is overcome at higher concentrations of 2-dGlc.

Effects of diphenylene iodonium on glucose-dependent superoxide production

Diphenylene iodonium (DPI) is an inhibitor of superoxide production in macrophages [26]. It acts by blocking NADPH oxidase activity [27], and hence prevents PMA-dependent superoxide production. The effect of varying concentrations of DPI on the PMA-stimulated glucose-dependent superoxide production is shown in Fig. 5. The maximal rate of superoxide production (control V_{max} = 64.37 ± 5.94 μmol/20 min per ml cell water) is reduced by 50% by 0.1 μM DPI, (V_{max} = 29.57 ± 0.67 μmol/20 min per ml cell water; $P < 0.001$) and by 95% by 1 μM DPI (V_{max} = 5.99 ± 0.44 μmol/20 min per ml cell water; $P < 0.001$). However,

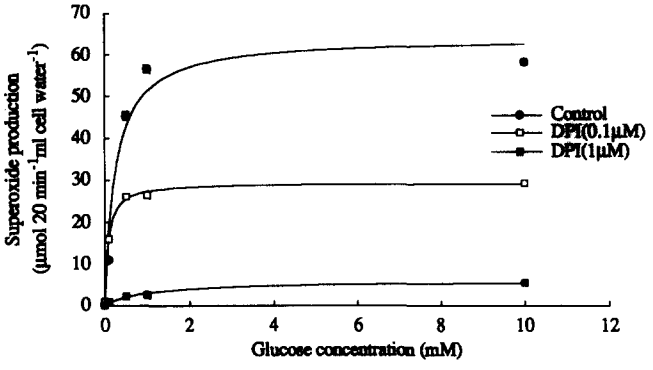

Fig. 5. Effects of diphenylene iodonium on glucose-dependent superoxide production. Peritoneal macrophages were pre-incubated with DPI (0.1 or 1 μM) for 60 min in sugar-free Hepes-buffered saline at 37°C then washed and transferred to buffer containing varying concentrations glucose (0–10 mM) with cytochrome *c* (20 μM) and PMA (40 nM). The production of superoxide was then measured over the next 20 min at 37°C (see Materials and Methods) and the results (means ± S.E.; μmol/20 min per ml cell water) processed using KaleidaGraph according to the legend for Fig. 2. The maximal rate of PMA-induced glucose-dependent superoxide production was significantly inhibited by both concentrations of DPI ($P < 0.001$, in both cases). DPI has no significant effect on the K_m of glucose-dependent superoxide production. Control, (●); DPI (0.1 μM), (□); DPI (1 μM), (■).

TABLE I

Effects of dexamethasone, PMA and DPI on 2-dGlc accumulation in macrophages

Peritoneal macrophages were incubated with or without dexamethasone (0.1 μM), PMA (40 nM) and DPI (1 μM) for 90 min at 37°C in Hepes-buffered saline containing 2-d[1-^{3}H]glucose (0.1 mM). The accumulated levels of total sugar, free sugar and hexose phosphates were determined by anion-exchange chromatography (see Materials and Methods) and the results (means ± S.E.) are expressed as μmol/90 min per ml cell water. PMA significantly increases steady-state accumulation of total and free sugar and 2-dGlc-6-*P* above control values ([a] $P < 0.001$ for total and free sugar; [b] $P < 0.05$ for 2-dGlc-6-*P*). Dexamethasone (0.1 μM) alone significantly decreases steady-state accumulation of total and free sugar and 2-dGlc-6-*P* below control values ([c] $P < 0.001$, in all cases). In PMA-treated cells, dexamethasone (0.1 μM) significantly decreases steady-state accumulation of total sugar (but not free sugar and 2-dGlc-6-*P*) when compared with the values with PMA alone, ([d] $P < 0.01$). DPI (1 μM) alone significantly increases steady-state accumulation of total and free sugar (but not 2-dGlc-6-*P*) above control values, ([e] $P < 0.001$ for total and free sugar). In PMA-treated cells, DPI (1 μM) significantly increases steady-state accumulation of free sugar (but not total sugar or 2-dGlc-6-*P*) when compared with the values with PMA alone ([f] $P < 0.01$). *n*, number of determinations; n.s., not significant.

	Control	PMA (40 nM)	Dexa (0.1 μM)	PMA + Dexa	DPI (1 μM)	PMA + DPI
Total	5.08 ± 0.07	6.85 ± 0.18 [a]	3.79 ± 0.08 [c]	5.88 ± 0.21 [d]	6.07 ± 0.07 [e]	6.80 ± 0.43 (n.s.)
Free	2.56 ± 0.12	3.30 ± 0.10 [a]	1.74 ± 0.08 [c]	3.08 ± 0.12 (n.s.)	3.54 ± 0.14 [e]	4.01 ± 0.24 [f]
Phos	2.29 ± 0.10	2.78 ± 0.15 [b]	1.58 ± 0.09 [c]	2.38 ± 0.16 (n.s.)	2.33 ± 0.13 (n.s.)	2.71 ± 0.31 (n.s.)
n	11	11	11	11	9	9

DPI has no significant effect on the K_m of glucose-dependent superoxide production (control $K_m = 0.26 \pm 0.10$ mM; 0.1 μM DPI $K_m = 0.28 \pm 0.11$ mM; 1 μM DPI $K_m = 0.32 \pm 0.16$ mM). DPI acts as a non-competitive inhibitor of superoxide production (K_i of DPI approx. 1 or 4.33 μM).

Effects of DPI on 2-dGlc uptake and accumulation

Control cells. DPI (1 μM) raises steady-state accumulation of total and free 2-dGlc (0.1 mM) when compared with the control condition ($P < 0.001$ in both cases; Table I), but has no significant effect on 2-dGlc-6-*P* accumulation. In addition, DPI (1 μM) produces a small but significant increase in c-2,6-labelled 2-dGlc uptake into macrophages when compared with control cells, ($P < 0.05$), but does not significantly affect the negligible HMPS activity in control cells (Table II).

PMA-treated cells. PMA (40 nM) significantly increases the rate of uptake of c-2,6-labelled 2-dGlc (0.1 mM), as shown in Table II, ($P < 0.001$); the uptake of c-2,6-labelled 2-dGlc (i.e., total sugar uptake) is increased by 56 ± 8%. However, when the concentration of 2-dGlc is $\geq$ 2 mM, PMA is without significant effect on 2-dGlc influx into macrophages [2].

The HMPS flux, as determined by the difference in net influx of c-1 and c-2,6-labelled 2-dGlc (0.1 mM; Table II) is significantly stimulated by PMA above the control condition; control HMPS = 0.11 ± 0.06 μmol/15 min per ml cell water ($n = 11$); PMA-induced HMPS = 1.26 ± 0.14 μmol/15 min per ml cell water ($n = 8$; $P < 0.001$). DPI (1 μM) has no significant ef-

TABLE II

Effects of DPI and PMA on 2-d[1-^{3}H]Glc and 2-d[2,6-^{3}H]Glc uptake and HMPS activity

Peritoneal macrophages were pre-incubated for 60 min with or without DPI (1 μM) in sugar-free saline at 37°C and then transferred into medium with or without PMA (40 nM) and containing either 2-d[1-^{3}H]Glc or 2-d[2,6-^{3}H]Glc (0.1 mM). Uptake of the labelled sugars was measured in parallel over 15 min (see Materials and Methods) at 37°C and the results (means ± S.E.) are expressed as μmol/15 min per ml cell water. Hexose monophosphate shunt (HMPS) activity was calculated from the difference in the uptake of 2-d[1-^{3}H]Glc and 2-d[2,6-^{3}H]Glc (see Materials and Methods). PMA (40 nM) significantly increased both the rate of uptake of c-2,6-labelled 2-dGlc and the HMPS activity above the control value ([a] $P < 0.001$, in both cases). DPI (1 μM) alone induces a small but significant increase in c-2,6-labelled 2-dGlc uptake when compared with control cells ([b] $P < 0.05$), but does not significantly affect the HMPS activity in control cells. In PMA-treated macrophages, DPI (1 μM) has no significant effect on the PMA-induced increase in c-2,6-labelled 2-dGlc uptake, but significantly reduces the activity of the HMPS ([c] $P < 0.01$).

2-dGlc (mM)	Isotope	Control	PMA (40 nM)	DPI (1 μM)	PMA + DPI
0.1	c-1	1.19 ± 0.04	0.77 ± 0.11	1.39 ± 0.04	1.58 ± 0.08
	c-2,6	1.30 ± 0.04	2.03 ± 0.08 [a]	1.45 ± 0.06 [b]	2.19 ± 0.12 (n.s.)
	HMPS	0.11 ± 0.06	1.26 ± 0.14 [a]	0.06 ± 0.07 (n.s.)	0.61 ± 0.14 [c]
n		11	8	6	6

fect on the PMA-induced increase in c-2,6-labelled 2-dGlc (0.1 mM) uptake into macrophages (Table II), but significantly reduces the activity of the HMPS to 0.61 ± 0.14 μmol/15 min per ml cell water ($n = 6$; $P < 0.01$).

Steady-state accumulation of both free sugar and 2-dGlc-6-*P* are increased significantly by PMA above the control condition, ($P < 0.001$ and $P < 0.05$, respectively; Table I); however, when 2-dGlc is raised to $\geqq 2$ mM PMA is without significant effect [2]. With DPI and PMA present the steady-state free sugar accumulation is significantly raised above that found in cells treated with PMA alone ($P < 0.01$), but there is no significant difference in the levels of total sugar and 2-dGlc-6-*P* in PMA- and PMA + DPI-treated macrophages (Table I).

Discussion

The results can be summarised as follows:

(a) The V_{max} of superoxide production is 10–12-fold less with 2-dGlc than with glucose and the K_m is also less with 2-dGlc (0.09 ± 0.01 mM) than with glucose (0.28 ± 0.08 mM).

(b) Dexamethasone reduces the V_{max} of PMA-stimulated glucose-dependent superoxide production without affecting the K_m; but in contrast, dexamethasone increases the K_m of 2-dGlc-dependent superoxide production in PMA-stimulated cells without affecting the V_{max}.

(c) DPI inhibits PMA-stimulated 2-dGlc-dependent HMPS and also glucose-dependent superoxide production non-competitively, but does not reduce the PMA-dependent increase in total 2-dGlc uptake. DPI increases free 2-dGlc accumulation in control and PMA-treated cells.

Explanation of the difference in macrophage response to PMA and dexamethasone with glucose and 2-dGlc

Hexokinase coupled to the endofacial surface of the cell membrane binds newly-transported hexose and transforms it to hexose-6-*P* within the cell cortex prior to its equilibration with the cytosolic pool. Hence, the cortical hexose concentration will be reduced by hexokinase action below that in the external bulk solution or within the core cytosol. As $[2\text{-dGlc}]_{free}$ accumulates within the cytosolic core, the cortical hexokinase becomes saturated and the efficiency of the hexokinase trap for newly-transported 2-dGlc reduced. With bulk solution concentrations of 2-dGlc > 1 mM, the accumulated cytosolic $[2\text{-dGlc}]_{free}$ saturates cortical hexokinase and hence, free 2-dGlc readily escapes from the cytosol across the membrane transport system; thereby reducing the efficiency of net uptake of 2-dGlc into the cell.

Because intracellular glucose is irreversibly and rapidly transformed by glycolysis, or via the HMPS, it does not accumulate to any great extent within the cytosol, so at higher external concentrations, i.e., > 1 mM, the rate of glucose net uptake is much higher than for 2-dGlc.

Dexamethasone, which reduces cortical hexokinase activity and hence reduces the capacity of the cells to accumulate 2-dGlc against a concentration gradient, raises the apparent K_m of net uptake of 2-dGlc, but does not alter the V_{max} of 2-dGlc uptake into PMA-activated cells. The explanation for the dexamethasone-induced increase in K_m is that the cortical hexokinase activity becomes saturated at a higher external [2-dGlc] than in controls, due to the reduced capacity to accumulate 2-dGlc within the cell. In contrast, with glucose, because there is no tendency for hexokinase to become saturated by intracellular glucose, dexamethasone inhibits superoxide production by reducing V_{max} of cortical hexokinase. At higher concentrations of glucose in the external solution, net uptake is rate-limited by saturation of the membrane transport system, hence the K_m of the glucose net uptake process is unaffected by dexamethasone.

PMA increases cortical hexokinase activity, and hence the tendency of free 2-dGlc to accumulate within the cytosolic core. As a result saturation of the cortical hexokinase will occur at a lower external concentration than with controls or with dexamethasone treatment. This will lead to an increase in the rate of 2-dGlc net uptake into macrophages at low external sugar concentrations, and to a reduction in the K_m of the process. With glucose as substrate, there will be an increase in uptake over a much wider concentration range than with 2-dGlc.

It should be noted that the K_m for 2-dGlc-dependent superoxide production is 0.09 ± 0.01 mM (Fig. 2) and with an external concentration of 0.1 mM 2-dGlc, free 2-dGlc accumulates within the cell to a concentration of 1–2 mM (Table I). The K_m of 2-dGlc for hexokinase has been reported to be about 100 μM [16]. The apparent insensitivity of endofacial hexokinase to accumulated $[2\text{-dGlc}]_{free}$ indicates that its concentration is lower (due to high hexokinase activity) in this region than in the remainder of the cytosol (see below).

There are 2 mol of superoxide produced per mol of NADPH utilised via the membrane-bound NADPH oxidase. Since 2 mol of NADPH are produced per mole of hexose-phosphate metabolised via the HMPS, it follows that the ratio of mol superoxide produced to mol sugar metabolized is 4:1. This ratio was observed for PMA-induced 2-dGlc-dependent superoxide production and HMPS metabolism in rat peritoneal macrophages [1] and indicates that the observed rate of superoxide production in these cells is close to the theoretical limit. As a result, superoxide production can be equated with HMPS activity with regard to 2-dGlc metabolism in rat peritoneal macrophages.

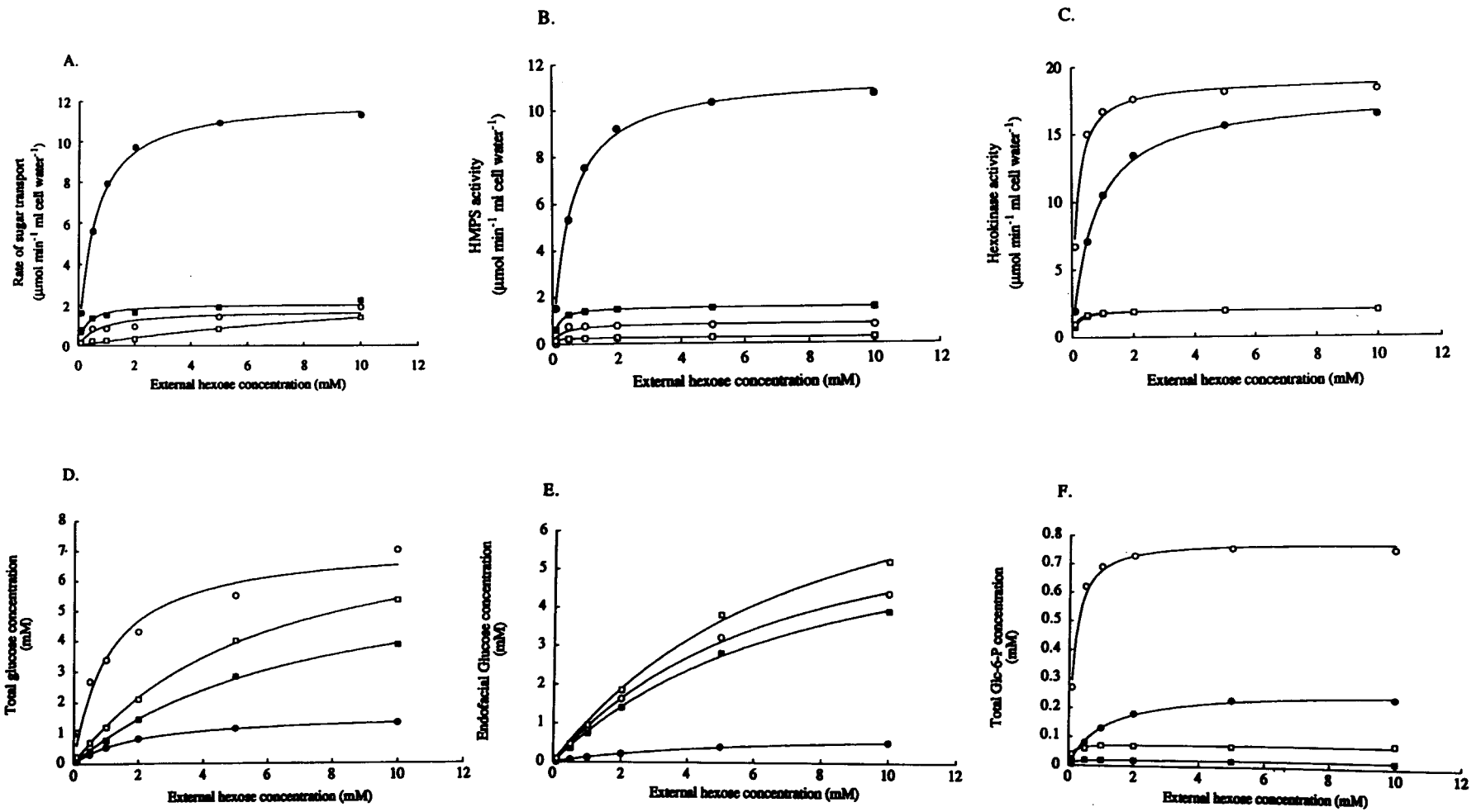

Fig. 6. Simulated data from the model. The rate of sugar transport (A, μmol/min per ml cell water), hexose monophosphate shunt activity (B, μmol/min per ml cell water), hexokinase activity (C, μmol/min per ml cell water), total glucose concentration (D, mM), endofacial glucose concentration (E, mM) and total glucose-6-phosphate (F, mM) are plotted against external hexose concentration (0–10 mM) for each of the four modes. Mode 1 (high hexokinase; high HMPS; PMA + Glc), (●); mode 2 (high hexokinase; low HMPS; PMA + 2-dGlc), (○); mode 3 (low hexokinase; high HMPS; PMA/Dexa + Glc), (■); mode 4 (low hexokinase; low HMPS; PMA/Dexa + 2-dGlc), (□).

Simulation of data with model

The principal reason for using a computer model to simulate the results is to show that the different cellular response to 2-dGlc or glucose in the presence of inhibitors or activators of superoxide production arises solely from the low rate of metabolism of 2-dGlc via the HMPS and the absence of its metabolism via the glycolytic pathway.

Four different parameter sets are used to illustrate the separate effects of 2-dGlc and glucose on superoxide production and of dexamethasone on stimulated hexose transport and superoxide production (Fig. 6). The K_m values as described in the model all refer to those apparent K_m values obtained on correlating the model parameter (either a rate, e.g., net transport; hexokinase activity or HMPS activity or an intracellular accumulation) against the external sugar concentration. Thus, in most cases where the operational K_m values are referred to, these will differ from the model K_m values which are assigned as function parameters and are fixed (Fig. 1).

In mode 1 (PMA-stimulated mode with glucose as substrate), the V_{max} of the endofacial hexokinase and HMPS are high and glycolysis is present; this leads to dissipation of Glc-6-*P* from the cytosolic pool. This mode simulates glucose transport and metabolism in PMA-stimulated cells where superoxide is generated.

In mode 2 (PMA-stimulated mode with 2-dGlc as substrate), the V_{max} of the membrane transporter and endofacial hexokinase are high, the same as with glucose (mode 1), but the V_{max} of the HMPS is low (5% of that with glucose) and glycolysis is absent; the K_m for 2-dGlc interaction with the membrane transporter and hexokinase are the same as for glucose. This mode simulates the effects of PMA on 2-dGlc-dependent transport and superoxide production.

In mode 3 (PMA and dexamethasone present with glucose as substrate), the V_{max} of the endofacial hexokinase is low (10% of that with control mode 1), but the V_{max} of the HMPS and glycolysis remain the same as in mode 1; this simulates the effects of dexamethasone on PMA-stimulated glucose transport and superoxide production.

In mode 4 (PMA and dexamethasone present with 2-dGlc as substrate), the V_{max} of endofacial hexokinase and of the HMPS are both lower (10 and 5%, respectively, than those in mode 1) and glycolysis is absent. This mode simulates the effect of dexamethasone on 2-dGlc-dependent transport and superoxide production.

In all cases shown the cells are activated; both HMPS activity and superoxide production are raised above the values expected for quiescent cells.

The major effects of changing from glucose to 2-dGlc as a substrate, i.e., comparison of mode 1 with mode 2 (Fig. 6; filled circles and open circles) are:

(i) A decrease in the apparent V_{max} of transport from 12.18 ± 0.19 to 1.74 ± 0.37 μmol/min per ml cell water without any change in K_t (approx. 0.6 mM).

(ii) A decrease in the apparent K_m of hexokinase from 0.71 ± 0.04 to 0.16 ± 0.02 mM; the real K_m of hexokinase for hexose in the endofacial compartment is 0.1 mM for both modes.

(iii) Reduction in the apparent V_{max} of the HMPS from 11.46 ± 0.19 to 0.87 ± 0.11 μmol/min per ml cell water with a concomitant reduction in K_m from 0.55 ± 0.04 to 0.26 ± 0.18 mM.

(iv) The total amount of free sugar accumulated within the first 2 min increases on going from mode 1 ($V_{max} = 1.59 \pm 0.02$ mM and $K_m = 2.00 \pm 0.08$ mM) to mode 2 ($V_{max} = 7.22 \pm 0.56$ mM; $K_m = 1.09 \pm 0.28$ mM); the ratio V_{max}/K_m changes from 0.80 (mode 1) to 6.6 (mode 2), i.e., by 8.3-fold.

(v) The total hexose phosphate accumulated is increased in mode 2 compared with mode 1; in mode 2 the $V_{max} = 0.79 \pm 0.01$ mM and $K_m = 0.17 \pm 0.02$ mM, whereas in mode 1 $V_{max} = 0.27 \pm 0.01$ mM and $K_m = 1.11 \pm 0.09$ mM; an increase in V_{max}/K_m ratio from 0.24 to 4.6 (19.2-fold).

The main reason for the differences between apparent rates of transport and hexokinase activity are due to accumulation of large amounts of free sugar concentration and unmetabolised hexose phosphate in the cell in mode 2. Even at low external sugar concentrations there is marked inhibition of 2-dGlc uptake; the V_{max}/K_m ratio which is equivalent to the highest obtainable permeability is only 11% of the ideal value. In mode 1, with high rates of glycolysis and HMPS, there is very little accumulation of free sugar in the endofacial layer and transport is hardly inhibited; the V_{max}/K_m ratio is 100% of the ideal value.

In this study, the model shows that in changing the substrate from mode 1 (PMA-stimulated; glucose substrate) to mode 2 (PMA-stimulated; 2-dGlc substrate), the V_{max} for HMPS activity is reduced by 92%, whilst the K_m is reduced by 53%. In addition, changing from mode 1 to mode 2 decreases the V_{max} of sugar transport by 86%, but does not significantly alter the K_m.

The observed results show that changing from glucose to 2-dGlc as a substrate reduces the V_{max} of PMA-stimulated superoxide production by 91%, whilst the K_m is reduced by 68% (Fig. 2). Thus, the predictions from the model for the effects of changing substrate from glucose to 2-dGlc on HMPS activity and sugar transport are closely correlated with the measured effects on PMA-stimulated superoxide production.

Simulation of the effects of dexamethasone

The effects of dexamethasone have been ascribed mainly to an inhibition of endofacial hexokinase activity [2].

Comparison of mode 1 with mode 3 (Fig. 6; filled circles and filled squares) simulates the inhibitory effect of dexamethasone on glucose-dependent rates of transport and superoxide production; comparison of mode 2 with mode 4 (Fig. 6; open circles and open squares) simulates the inhibitory effect of dexamethasone on 2-dGlc-dependent transport and superoxide production. In both modes 3 and 4 (dexamethasone), the V_{max} of endofacial hexokinase activity is reduced to 2 μmol/min per ml of cells, whereas in mode 1 and 2 (controls) it is 20 μmol/min per ml cell water, i.e., dexamethasone is assumed to reduce the V_{max} of cortical hexokinase activity by 90% without affecting the real K_m of hexokinase.

The major observed differences between the simulations of modes 1 and 3 (Fig. 6; filled circles and filled squares) are:

(1) The V_{max} of transport falls from 12.18 ± 0.19 (mode 1) to 2.07 ± 0.13 μmol/min per ml cell water (mode 3) and the K_m falls from 0.57 ± 0.04 to 0.28 ± 0.09 mM; thus, at high concentrations the transport rate of glucose falls by 83% after exposure to dexamethasone. At low concentrations, e.g., 0.1 mM external glucose the V_{max}/K_m ratio for transport gives 21.4 for mode 1 and 7.39 for mode 3, i.e., a decrease of only 65%. These values are reasonably close to the ideal values which should be obtained if intracellular glucose concentrations have no influence on transport.

(2) The effect of dexamethasone on the HMPS pathway is similar to that on transport; there is a fall in V_{max} from 11.46 ± 0.19 (mode 1) to 1.59 ± 0.01 μmol/min per ml cell water (mode 3); the apparent K_m of the HMPS also falls from 0.55 ± 0.04 (mode 1) to 0.15 ± 0.01 mM (mode 3); hence at high concentrations of external glucose there is an 86% decrease in HMPS activity; at low concentrations of external sugar (0.1 mM) the decrease in HMPS activity is 49%.

(3) The apparent rate of hexokinase activity falls from a V_{max} of 17.74 ± 0.27 to 2.0 ± 0.02 μmol/min per ml cell water; a decrease of 89% (ideal fall 90%).

The model predicts that dexamethasone should give an 83% decrease in the V_{max} of glucose transport and as glucose transport is the rate determining step, an 86% decrease in the V_{max} of glucose-dependent HMPS activity follows. This finding is consistent with the measured effects of dexamethasone (0.1 μM) on glucose-dependent superoxide production (Fig. 3), which induces a 59% decrease in the V_{max} of the process.

The major differences between modes 2 and 4 (Fig. 6; open circles and open squares) (effects of dexamethasone on 2-dGlc-dependent transport and superoxide production in PMA-stimulated cells) are:

(1) The V_{max} for transport in mode 2 is 1.74 ± 0.37 and in mode 4 is 3.76 ± 1.7 μmol/min per ml cell water, i.e., not significantly different. The K_m (mode 2, 2-dGlc, control) = 0.79 ± 0.63 and K_m (mode 4, 2-dGlc with dexamethasone) = 17.22 ± 11.00 mM. The V_{max}/K_m ratio changes from 2.2 (mode 2) to 0.22 (mode 4), a reduction of 90%.

(2) The V_{max} for HMPS (mode 2) is 0.87 ± 0.11 and in mode 4 0.26 ± 0.01 μmol/min per ml cell water; the K_m in mode 2 is 0.26 ± 0.18 and in mode 4 0.01 ± 0.01, hence at high concentrations of external 2-dGlc, dexamethasone reduces the HMPS by 70%.

(3) The V_{max} of hexokinase falls from 18.89 ± 0.33 (mode 2) to 1.98 ± 0.05 μmol/min per ml cell water (mode 4); a reduction of 90%, which is equal to the ideal value.

(4) Dexamethasone reduces the accumulation of both total and free 2-dGlc and total cell 2-dGlc-6-*P*; the effect on free 2-dGlc accumulation is seen best at low external [hexose].

The model predicts that treatment of macrophages with dexamethasone should not alter the V_{max} of 2-dGlc transport. This finding is consistent with the measured effects of dexamethasone (0.1 μM) on 2-dGlc-dependent superoxide production (Fig. 4), where no significant effect on the V_{max} of the process is observed. Additionally, the model simulates a dexamethasone-dependent increase in the K_m of 2-dGlc transport [2] which is paired with a dexamethasone-induced increase in the K_m of 2-dGlc-dependent superoxide production (Fig. 4). Thus the predictions of the model for the effects of dexamethasone on 2-dGlc transport are similar to the measured effects of dexamethasone on PMA-stimulated 2-dGlc-dependent superoxide production.

In conclusion, it is clear that the model simulates the apparent major kinetic differences between glucose and 2-dGlc-dependent transport, HMPS activity and superoxide production seen in rat macrophages. Moreover, it has been demonstrated that these apparent differences can be explained on the basis of known differences in the metabolism of these sugars.

Interpretation of the effects of RU 38486

It has been reported that RU 38486 has powerful anti-glucocorticoid activity in vivo and in vitro [24] and that it exerts its antagonistic effects by binding to the cytosolic glucocorticoid receptor [25]. The data reported here show that treatment of macrophages with RU 38486 (1 μM) reverses the non-competitive inhibition of PMA-stimulated glucose-dependent superoxide production by dexamethasone (0.01 μM; Fig. 3b). This finding is consistent with a receptor-mediated blocking of the dexamethasone-dependent inhibition by RU 38486 and emphasises the specificity of the effects of dexamethasone on activated macrophages.

Interpretation of the effects of DPI

DPI inhibits superoxide production by inhibiting NADPH oxidase activity [27] and it is apparent that

DPI has no inhibitory action on the PMA-dependent increase in endofacial hexokinase activity. In Table II, the major effect of DPI is to reduce the PMA-dependent increase in 2-dGlc-dependent HMPS activity from 1.26 ± 0.14 to 0.61 ± 0.14 μmol/15 min per ml cell water ($P < 0.01$) without significantly affecting the PMA-dependent increase in c-2,6-labelled 2-dGlc uptake. In Table I, it can be seen that DPI has no significant effect on steady-state accumulation of 2-dGlc in PMA-treated cells, although it does increase sugar accumulation in control cells ($P < 0.01$).

These results are additional evidence supporting the view that the PMA-dependent increase in hexose transport is controlled independently from superoxide metabolism. There is no significant feedback control between the HMPS and 2-dGlc transport, i.e., the increased metabolic demand of an activated HMPS is not a sufficient stimulus to increase sugar uptake significantly. This may reflect the fact that 2-dGlc-6-*P* metabolism via the HMPS is much slower than total hexokinase and phosphatase activity within the cell.

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