Effects of phorbol, dexamethasone and starvation on 3-*O*-methyl-D-glucose transport by rat thymocytes

Modulation of transport by altered *trans* effects

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Uptake of 3-*O*-methyl-D-glucoside (3-OMG) into thymocytes was studied to ascertain if it is modulated by endofacial hexokinase activity or by intracellular glucose. (1) The $V_{\max}$ for net uptake of 3-OMG into rat thymocytes is increased by phorbol 12-myristate 13-acetate (PMA; 40 nM) or starvation for 4 h, and decreased by dexamethasone (1 μ M). Starvation for 4 h abolishes the PMA-dependent increase in 3-OMG uptake; this effect is prevented by incubation in 2-deoxyglucose (2-dGlc; 1 mM). (2) Dexamethasone decreases 2-dGlc uptake, increases the rate of 2-dGlc exit and decreases accumulation of free 2-dGlc, consistent with decreased endofacial hexokinase activity. (3) 3-OMG uptake is decreased by preloading the cells with 2-dGlc or glucose, whereas preloading with 3-OMG (40 mM) increases uptake of 3-OMG. (4) The inhibitory effect of preloaded 2-dGlc or glucose on 3-OMG uptake is decreased by PMA. (5) Preloading cells with 3-OMG (40 mM) increases 2-dGlc influx in control and dexamethasone-treated cells, but not into PMA-treated cells. (6) The maximal rate of self-exchange of 3-OMG is similar in control, PMA- or dexamethasone-treated cells. These results are consistent with the following view: 3-OMG uptake is retarded by exchange with cytosolic glucose, or 2-dGlc. PMA, by increasing endofacial hexokinase activity, or starvation depletes glucose from the endofacial surface of the transporter, and hence increase 3-OMG uptake. Dexamethasone, by decreasing endofacial hexokinase activity, increases endofacial binding of glucose, and hence decreases 3-OMG uptake. Cytosolic 3-OMG competes with glucose for endofacial sites, and hence the maximal rates of exchange uptake of 3-OMG are similar in control, PMA- or dexamethasone-treated cells, as the activity of thymocyte glucose transporters is apparently unaltered.

INTRODUCTION

The rate of uptake of 2-deoxy-D-glucose (2-dGlc) and its activation by phorbol 12-myristate 13-acetate (PMA) are coupled to hexokinase activity at the endofacial surface of rat thymocytes and Chinese-hamster ovary cell membranes (Naftalin & Rist, 1989; Faik *et al.*, 1989). Loose coupling between transport and hexokinase activity also permits intracellular accumulation of the free 2-dGlc above the concentration in the external bathing solution and retards exit of cytosolic free sugar (Naftalin & Smith, 1987).

The rate of uptake of the non-metabolized sugar 3-*O*-methyl-D-glucoside (3-OMG) into rat thymocytes and other single-cell systems is also stimulated by PMA (Whitesell & Regen, 1978; Gay & Hilf, 1980; O'Brian, 1982; Nordenberg *et al.*, 1983; Lemmon *et al.*, 1985; Van Putten & Krans, 1985; Haspel *et al.*, 1986).

Depriving cells of glucose (starvation) increases the rate of uptake of 3-OMG and of 2-dGlc by increasing the $V_{\max}$ for net uptake 2–40-fold without greatly affecting the K_m (Christopher *et al.*, 1976; Gay & Hilf, 1980; Lemmon *et al.*, 1985; Van Putten & Krans, 1985; Haspel *et al.*, 1986; D'Amore *et al.*, 1986; Germinario *et al.*, 1989), and in contrast glucocorticoids such as dexamethasone decrease the rates of 3-OMG, 2-dGlc and Glc

uptake into thymocytes, adipocytes and fibroblasts by decreasing the $V_{\max}$ (Hallahan *et al.*, 1973; Carter-Su & Okamoto, 1985*a,b*; Horner *et al.*, 1987).

These effects of starvation, PMA or dexamethasone on 3-OMG uptake cannot be easily reconciled with the view that sugar uptake is solely modulated by altered coupling of transport with the hexokinase reaction, as 3-OMG is not a substrate for the hexokinase reaction. The conventional explanation of the changes in uptake of 3-OMG is that they are related to the number or activity of sugar transporters in the cell membrane, owing to new protein synthesis (Christopher *et al.*, 1976; Gay & Hilf, 1980; Haspel *et al.*, 1986). Although there is good evidence for an enhanced number of glucose transporters in the plasma membrane of adipocytes after insulin treatment or starvation of adipocytes or fibroblasts (Lo & Duronio, 1984; Gibbs *et al.*, 1986; Haspel *et al.*, 1986; Simpson & Cushman, 1986) and a decrease after glucocorticoids, it is also apparent that a change in the number of transporters within the cell membrane is not the sole mode of control of transport activity, as the number of sugar transporters within the cell membrane and transport activity are not always correlated, e.g. after treatment with inhibitors of protein synthesis (Whitesell & Abumrad, 1985; Joost *et al.*, 1986; Greco-Perotto *et al.*, 1987; Matthaei *et al.*, 1988).

The capacity of thymocytes to accumulate intracellular free sugar, i.e. 2-dGlc or Glc, to a higher concentration than is present in the external medium raises the possibility that free intracellular sugar could modify the rate of uptake of labelled sugar into the cells, either by *trans*-acceleration of uptake, or *trans*-inhibition, as has been observed previously with hetero-exchanges of sugars in human red cells (Naftalin, 1971) or with nucleosides (Jarvis, 1986). If hetero-exchange of sugars is slower than net influx of labelled sugar, then the presence of a retarding sugar within the cytosol will act as a non-competitive inhibitor of labelled sugar influx. This effect will be kinetically indistinguishable from a change in the number of sugar transporters within the cell membrane. The slow rate of sugar transport, together with the capacity of thymocytes to accumulate actively and retain free sugars within the cytosol, permits the investigation of the effects of preloading the cytosolic compartment with high concentrations of unlabelled sugars on subsequent uptake of labelled sugar from fresh external solution (infinite-*trans* effects on hetero- or auto-exchange uptake: Lieb & Stein, 1972; Naftalin & Holman, 1977).

In the present paper the effects of dexamethasone on 2-dGlc transport and accumulation by rat thymocytes and the effects of PMA and dexamethasone on uptake of 3-OMG into cells loaded with zero sugar, into starved cells, or into cells preloaded with unlabelled 3-OMG, 2-dGlc or glucose are reported.

METHODS

Cell preparation

Preparation of rat thymocyte suspensions was as previously described (Naftalin & Rist, 1989). Thymus glands were removed from Wistar rats weighing 120–200 g and immersed in Hepes-buffered saline (Hepes 5 mM, NaCl 140 mM, KCl 5 mM, MgCl₂ 1.2 mM, pH 7.3) and chopped into small pieces. Disaggregation was then effected by repeated cycles of suction and extrusion with a 10 ml syringe. After removal of any debris from the cell suspension, the cells were centrifuged at 400 *g* for 1 min to precipitate aggregates, and the supernatants were decanted and re-centrifuged for 3 min at 2000 *g* to concentrate the cells.

Microscopy and cell counting

This method of disaggregation does not cause discernible damage to the appearance or integrity of the cells. There is very little cell debris in the preparations, and it is hard to detect any dead cells by Evans Blue uptake.

All sugar uptake rates and estimates of intracellular concentrations of free sugar and hexose phosphates are normalized to 10¹⁰ cells. As the mean cell volume is approx. 100 fl, 10¹⁰ cells are equivalent to approx. 1 ml of cell water. The cell numbers were measured with a Coulter Counter model B. The concentration of cells in the suspension used for influx was approx. 5 × 10⁶/ml and that for efflux (1–2) × 10⁷/ml.

Activation with PMA

Before uptake measurements, the cell suspensions were preincubated for 30 min in solutions containing PMA (40 nM) or in control solutions. This concentration of

PMA was found to give optimal activation over a prolonged incubation. Similarly, it was found that preincubation of rat thymocytes for 30 min with 1 μM-dexamethasone gave a satisfactory and reproducible inhibition of net 2-dGlc and 3-OMG uptake, and therefore these concentrations are used throughout this paper.

Radioactive-sugar uptake studies

Uptake of sugars into the cells was measured by using 2-deoxy-D-[1-³H]glucose (Amersham; initial sp. radioactivity 11.5 Ci/mmol) at a final radioisotope concentration of 0.2 μCi/ml, or 3-*O*-[U-¹⁴C]methylglucose (Amersham; initial sp. radioactivity 74.2 mCi/mmol), at a final radioisotope concentration of 0.05 μCi/ml.

After incubation, sugar uptakes were halted by adding iso-osmotic ice-cold stopping solution to the cell suspensions. This contained enough phloretin and HgCl₂ to give final concentrations of 100 μM and 1 μM respectively in the cell suspensions. The cell suspensions were centrifuged in a bench centrifuge for 2 min at 3000 *g*. The supernatants were removed, and a sample was retained for measurement of extracellular radioactivity (c.p.m.). The cells were washed twice more by suspension and re-centrifugation at 3000 *g* in 4 ml of stopping solution to remove extracellular label. Finally, the cell pellets were lysed in 4 ml of ice-cold distilled water. This lysate was used to determine the total uptake, free sugar and hexose phosphate concentrations within the cell pellet as previously described (Naftalin & Rist, 1989).

Studies on the effects of dexamethasone on the radioactive sugar exit from preloaded cell suspensions

These exit experiments were carried out as described previously (Naftalin & Rist, 1989). Suspensions containing approx. 2 × 10⁷ cells/ml were used in the preincubation solutions. Cells were incubated with ³H-labelled 2-dGlc (0.1 mM) for 2 h in control conditions. At the end of the incubation period, the cells were loosely packed by centrifugation at 1500 *g* for 3 min and the supernatant was removed. The cell pellet was suspended in Hepes buffered-saline at 20 °C, and the cells were immediately re-centrifuged at room temperature for 30 s. This washed cell pellet was diluted by approx. 50-fold by addition to fresh solution containing nominally zero sugar at 37 °C (2-dGlc < 0.02 mM).

Zero-*trans* net exit was measured by observing the time-dependent loss of radioactivity from the cell sugar and hexose phosphate pools into nominally sugar free solutions.

RESULTS

Effects of dexamethasone (1 μM) on 2-dGlc uptake

The concentration-dependence of 2-dGlc uptake into thymocytes incubated for 1 h in buffered saline, with or without dexamethasone (1 μM) added, is shown in Fig. 1. Each point is the mean of five separate determinations ± S.E.M. Estimates of the Michaelis-Menten parameters are $V_{\max} = 0.45 \pm 0.02$ μmol/min per 10¹⁰ cells and $K_m = 3.9 \pm 0.55$ mM. In the presence of dexamethasone, $V_{\max} = 0.3 \pm 0.06$ μmol/min per 10¹⁰ cells, which is significantly less than for control ($P < 0.05$), and $K_m = 3.0 \pm 1.6$ mM, which is not significantly different from control. These results are similar to those reported previously by Hallahan *et al.* (1973) and

Fig. 1. Concentration-dependent uptakes of 2-dGlc: ■, control; ●, dexamethasone (1 μM)

Lines fitted are the least-squares best fits to the Michaelis-Menten plots (Enzfitter). Control: $V_{max} = 0.45 \pm 0.02 \mu\text{mol/min per } 10^{10} \text{ cells}$; $K_m = 3.9 \pm 0.55 \text{ mM}$. Dexamethasone: $V_{max} = 0.3 \pm 0.06 \mu\text{mol/min per } 10^{10} \text{ cells}$ ($P < 0.05$); $K_m = 3.0 \pm 1.6 \text{ mM}$ ($n = 5$ for each point). Where no error bars are shown, the size of the symbol is larger than S.E.M.

Nordenberg *et al.* (1983), who also showed that dexamethasone inhibits uptake of 2-dGlc into thymocytes.

Dexamethasone caused a significant decrease in accumulation of total sugar, free sugar and hexose phosphate (Table 1), and PMA, as previously reported (Naftalin & Rist, 1989), significantly increased accumulation of total and free 2-dGlc and hexose phosphate above that found in controls. The accumulations of 2-dGlc in control and PMA-treated cells are lower than previously reported, because here the cells were incubated for 2 h with radioisotope instead of 3 h.

Effects of PMA (40 nM) and dexamethasone (1 μM) on the concentration-dependent uptake of 3-OMG

Uptakes of 3-OMG into control thymocytes, or

Fig. 2. Concentration-dependent 2 min uptakes of 3-OMG

●, Control ($n = 8$); ▲, PMA (40 nM) ($n = 8$); ■, dexamethasone (1 μM) ($n = 6$). Lines are best-fit non-linear least-square fits to the Michaelis-Menten equation (Enzfitter). Control: $V_{max} = 1.65 \pm 0.13 \mu\text{mol/2 min per } 10^{10} \text{ cells}$; $K_m = 6.4 \pm 1.6 \text{ mM}$. PMA: $V_{max} = 2.84 \pm 0.39 \mu\text{mol/2 min per } 10^{10} \text{ cells}$ ($P < 0.05$); $K_m = 9.19 \pm 3.64 \text{ mM}$. Dexamethasone: $V_{max} = 1.13 \pm 0.15 \mu\text{mol/2 min per } 10^{10} \text{ cells}$ ($P < 0.05$); $K_m = 5.22 \pm 2.5 \text{ mM}$. PMA increases V_{max} for 3-OMG uptake above control and dexamethasone decreases below control; there were no significant effects of PMA or dexamethasone on K_m of 3-OMG uptake. Where no error bars are shown, the size of the symbol is larger than S.E.M.

cells pretreated for 1 h with either PMA (40 nM) or dexamethasone (1 μM), are shown in Fig. 2. The Michaelis-Menten parameters for controls are $V_{max} = 1.65 \pm 0.13 \mu\text{mol/2 min per } 10^{10} \text{ cells}$ and $K_m = 6.4 \pm 1.6 \text{ mM}$; in PMA-treated cells $V_{max} = 2.84 \pm 0.39 \mu\text{mol/2 min per } 10^{10} \text{ cells}$, which is significantly above that of control ($P < 0.05$) and $K_m = 9.19 \pm 3.64 \text{ mM}$, which is not significantly different from control. Uptake into dexamethasone-treated tissue shows $V_{max} = 1.13 \pm 0.15 \mu\text{mol/2 min per } 10^{10} \text{ cells}$,

Table 1. Effects of dexamethasone (1 μM) and PMA (40 nM) on total and free sugars and hexose phosphate accumulation within 1×10^{10} thymocytes after 2 h incubation in 2-dGlc (0.1 mM)

*** $P < 0.001$, ** $P < 0.01$ for test versus control value on same row (Student's *t* test); n = number of separate determinations. Results are means $\pm$ S.E.M. PMA increases steady-state accumulation of total and free 2-dGlc and hexose phosphate above control. Dexamethasone decreases steady-state accumulation of total and free 2-dGlc and hexose phosphate below control.

	Accumulation ($\mu\text{mol}/10^{10} \text{ cells}$)		
	Control	PMA (40 nM)	Dexamethasone (1 μM)
Total	1.02 ± 0.09	2.13 ± 0.16 ***	0.54 ± 0.07 ***
Free sugar	0.56 ± 0.06	1.11 ± 0.08 ***	0.26 ± 0.03 ***
Hexose phosphate	0.54 ± 0.05	1.02 ± 0.11 **	0.27 ± 0.04 ***
n...	7	5	8

which is significantly less than that observed in controls ($P < 0.05$), but K_m (5.22 ± 2.5 mM) is unaffected by dexamethasone. These results are similar to those previously reported by Whitesell & Regen (1978) and Nordenberg *et al.* (1983), and show that, although PMA increases the rate of uptake of both 2-dGlc and 3-OMG, it does so by raising the V_{max} of 3-OMG uptake, whereas it decreases the K_m for net uptake of 2-dGlc into thymocytes, while leaving the V_{max} unaffected (Naftalin & Rist, 1989).

Effects of dexamethasone on 2-dGlc exit from thymocytes

Exposure of thymocytes to PMA retards free 2-dGlc exit without affecting 3-OMG exit (Naftalin & Rist, 1989). In this experiment cells were preloaded with 2-dGlc (0.1 mM), with or without dexamethasone, for 2 h. Accumulation of total and free sugar and hexose phosphate in the cells was determined as described in the Methods section.

Exit of free sugar was then monitored by determining the loss of total radioactivity from the cells during the ensuing 30 min. The decrease in cell sugar is expressed in terms of the percentage total loss of radioactivity, taking the zero-time counts for each condition as the reference. The linear regression line of the log (percentage loss of 2-dGlc) versus time shows that the rate of 2-dGlc exit is increased from $-0.0056 \pm 0.0001 \text{ min}^{-1}$ (control, $n = 7$) to $-0.0110 \pm 0.0008 \text{ min}^{-1}$ ($P < 0.001$) by dexamethasone (1 μM) (Fig. 3). This result shows that the decreased accumulation of free 2-dGlc in dexamethasone treated cells is due to both a decreased rate of uptake and an increased rate of exit of free sugar.

Fig. 3. Fractional loss of total sugar from control cells and cells treated with dexamethasone (1 μM)

The slope of the linear regression line of control exit = $-0.0056 \pm 0.0001 \text{ min}^{-1}$ (■) and the intercept = 1.999 ± 0.002 . For exit from dexamethasone-treated cells (●) the slope of the regression line = $-0.011 \pm 0.0008 \text{ min}^{-1}$ ($n = 7$) ($P < 0.001$) and the intercept = 2.000 ± 0.009 (100%) ($n = 8$). Where no error bars are shown, the size of the symbol is larger than S.E.M.

Effects of starvation and PMA on 2-dGlc and 3-OMG uptake

To test whether the PMA-dependent stimulation of 3-OMG transport is related to the starvation-induced changes in sugar transport, the effect of prolonged incubation either in glucose-free buffer or in buffer containing 2-dGlc (1 mM) on the PMA-dependent increment in 3-OMG or 2-dGlc uptake into thymocytes was observed.

(a) **Uptake of 3-OMG.** The cells were incubated in sugar-free solutions until the time set for incubation with radioactive sugar, when they were transferred to fresh solutions containing only labelled 3-OMG (10 mM) with or without PMA (40 nM).

The ratio of the rate of 3-OMG (10 mM) uptake (control/PMA-treated cells) into cells incubated for the times shown on the abscissa in sugar-free solution or with unlabelled 2-dGlc (1 mM) present is shown in Fig. 4. The initial control uptake rate was 2.7 ± 0.1 ($n = 5$) $\mu\text{mol}/5 \text{ min}$ per 10^{10} cells and with PMA present the uptake rate was 4.1 ± 0.2 ($n = 5$) $\mu\text{mol}/5 \text{ min}$ per 10^{10} cells ($P < 0.01$). However, after 4 h incubation in sugar-free solution, there was no significant difference between the initial rates of 3-OMG uptake into control cells and

Fig. 4. Ratio (control/PMA-treated cells) of the rate of 3-OMG (10 mM) uptake during 5 min into cells incubated for time shown on the abscissa either in sugar-free buffered saline (●) or with 1 mM unlabelled 2-dGlc present continuously (▲)

The initial control uptake rate was 2.7 ± 0.1 ($n = 5$) $\mu\text{mol}/5 \text{ min}$ per 10^{10} cells, and with PMA present the uptake rate was 4.1 ± 0.2 ($n = 5$) $\mu\text{mol}/5 \text{ min}$ per 10^{10} cells ($P < 0.01$). After 4 h incubation in sugar-free solution there was no significant difference between the initial rates of 3-OMG uptake into control cells and cells incubated with PMA [ratio control/PMA 1.10 ± 0.08 ($n = 5$)]. However, in the cells incubated with 2-dGlc (1 mM), 3-OMG uptake into PMA-treated cells is greater than control after 4 h [ratio control/PMA = 0.82 ± 0.02 ($n = 5$; $P < 0.01$)]. Where no error bars are shown, the size of the symbol is larger than S.E.M.

Table 2. Uptake of 2-dGlc (0.1 mM) into cells exposed to medium containing zero sugar with or without PMA (40 nM) or 2-dGlc (1 mM) with or without PMA (40 nM) for 30 min or 4 h

Each point represents the mean of five separate experiments. Starvation has no significant effect on the PMA-dependent increase in 2-dGlc uptake during the 4 h incubation. Preincubation of the cells in 2-dGlc (1 mM) for 30 min decreases the PMA-dependent increase in 2-dGlc uptake ($P < 0.01$), and after 4 h abolishes the PMA-dependent increase in 2-dGlc uptake. Results are means $\pm$ S.E.M. ($n = 5$): * $P < 0.05$, *** $P < 0.001$, test versus control zero 2-dGlc same time; ++ $P < 0.01$, +++ $P < 0.001$, test versus same condition at 30 min (ns, not significant).

Incubation time	Preloading concn. of 2-dGlc...	Uptake ($\mu\text{mol}/10 \text{ min pr } 10^{10} \text{ cells}$)			
		Control		Phorbol	
		0	1 mM	0	1 mM
30 min		0.12 ± 0.01	$0.08 \pm 0.01^*$	$0.22 \pm 0.02^{***}$	0.13 ± 0.01
4 h		$0.19 \pm 0.01^{+++}$	$0.10 \pm 0.01^{***,ns}$	$0.30 \pm 0.01^{***,++}$	$0.11 \pm 0.01^{***,ns}$

cells incubated with PMA (ratio of rates of 3-OMG uptake = 1.10 ± 0.08 ; $n = 5$). When the cells were incubated with 2-dGlc (1 mM), the PMA-dependent increase in 3-OMG uptake above control cell uptake was maintained for at least 4 h (ratio of 3-OMG uptake, control/PMA = 0.82 ± 0.02 ; $n = 5$, $P < 0.01$). This result indicates that the PMA-dependent increment in 3-OMG influx is abolished by prolonged incubation in sugar-free solution, but is maintained by the presence of the partially metabolized sugar 2-dGlc in the bathing solution.

(b) Effects of starvation and PMA on 2-dGlc uptake.

Uptake of ^3H -labelled 2-dGlc (0.1 mM) was determined into cells incubated in buffered saline, with or without unlabelled 2-dGlc (1 mM) present during the 4 h preincubation period. During the period of labelled-sugar uptake, the cells were incubated in fresh medium containing only labelled 2-dGlc (0.1 mM) with or without PMA (40 nM).

Starvation had no significant effect on the PMA-dependent increase in 2-dGlc uptake. After the 4 h incubation in sugar-free solutions, there was still a PMA-dependent increase in the rate of 2-dGlc uptake ($P < 0.001$) (Table 2). This contrasts with the starvation-induced loss of the PMA-dependent increase in 3-OMG uptake during this time (cf. Fig. 4). Preincubation of the

cells in 2-dGlc (1 mM) for 30 min decreased the PMA-dependent increase in 2-dGlc uptake ($P < 0.01$), and after 4 h abolished the PMA-dependent increase in 2-dGlc uptake entirely. This result differs from thymocyte transport of 3-OMG [cf. Fig. 4, where preincubation in 2-dGlc (1 mM) conserved the PMA-dependent increase in the rate of 3-OMG uptake].

These results indicate that, although both 2-dGlc and 3-OMG uptakes are increased by PMA and starvation, the processes involved in these increments are not identical. The PMA-dependent increase in 2-dGlc uptake is more resistant to the effects of starvation than is the PMA-dependent increase in 3-OMG uptake; also, the PMA-dependent increase in 3-OMG uptake is retained during preincubation with 2-dGlc (1 mM), whereas preincubation with 2-dGlc (1 mM) abolishes the PMA-dependent increase in 2-dGlc uptake. The results in this section indicate that changes in intracellular concentrations of accumulated sugars may alter the thymocyte response to PMA with respect to both 2-dGlc and 3-OMG transport.

Effects of preloading with 2-dGlc on uptake of 3-OMG

The capacity of cells to accumulate and retain free sugars within the cytosol above the concentration in the external solution raises the possibility that the rate of

Table 3. Effects of dexamethasone (1 μM) and 2-dGlc and glucose pre-loading on 3-OMG (10 mM) uptake in 5 min

Results are expressed as means $\pm$ S.E.M. for the numbers of independent observations in parentheses: ** $P < 0.01$, *** $P < 0.001$, test versus control zero 2-dGlc preload, ns refers to absence of a significant difference between test and dexamethasone zero 2-dGlc preload. Cells were preloaded with or without unlabelled 2-dGlc or Glc for 60 min and then transferred into fresh medium containing ^{14}C -labelled 3-OMG (10 mM). Uptake was stopped after 5 min. All results have been corrected for phloretin-insensitive uptake (approx. $0.2 \mu\text{mol}/5 \text{ min per } 10^{10} \text{ cells}$). 2-dGlc and glucose inhibit 3-OMG uptake in control, but not in dexamethasone-treated, cells.

Sugar	Preload Concn. (mM)	Uptake ($\mu\text{mol}/5 \text{ min per } 10^{10} \text{ cells}$)	
		Control	Dexamethasone
2-dGlc	0	3.07 ± 0.14 (10)	2.33 ± 0.10 (10)***
	1	2.44 ± 0.11 (7)**	2.20 ± 0.17 (7) ^{ns}
Glucose	10	2.23 ± 0.04 (4)***	2.17 ± 0.13 (4) ^{ns}

Table 4. Effects of PMA (40 nM) and 2-dGlc or glucose preloading on 3-OMG (10 mM) uptake in 5 min

Results are expressed as the means $\pm$ S.E.M. for the numbers of independent observations in parentheses: *, $\dagger P < 0.05$, **, $\dagger\dagger P < 0.01$, ***, $\dagger\dagger\dagger P < 0.001$; * refers to test versus control (zero preloading); $\dagger$ refers to test versus PMA (with zero preloading); ns (not significant) refers to test versus control preloaded with Glc (10 mM). Cells were preloaded with or without unlabelled 2-dGlc or glucose for 60 min and then transferred into fresh medium containing ^{14}C -labelled 3-OMG (10 mM). Uptake was stopped after 5 min. All results have been corrected for phloretin-insensitive uptake (approx. $0.2 \mu\text{mol}/5 \text{ min per } 10^{10}$ cells).

Preload		Uptake ($\mu\text{mol}/5 \text{ min per } 10^{10}$ cells)	
Sugar	Concn. (mM)	Control	PMA
2-dGlc	0	2.97 ± 0.10 (8)	3.97 ± 0.11 (8)***
	1	2.72 ± 0.06 (8)*	3.40 ± 0.09 (6)**, $\dagger\dagger$
	10	2.03 ± 0.06 (8)***	2.34 ± 0.07 (8)***, $\dagger\dagger\dagger$
Glucose	10	2.23 ± 0.04 (4)***	2.46 ± 0.20 (4)*, $\dagger\dagger\dagger$, ^{ns}

labelled sugar uptake could be modulated by *trans*-effects of the accumulated free sugar. Tables 3 and 4 show the effects of pre-loading thymocytes with 2-dGlc or glucose on the subsequent uptake of 3-OMG and the way PMA and dexamethasone interact with the sugar-transport system. In control cells, preloading with 2-dGlc or glucose decreased uptake of 3-OMG. A similar result has been noted previously in adipocytes and has been ascribed to the effect of increased glucose 6-phosphate (Foley & Huecksteadt, 1984), which they claim lowers the V_{max} for entry of 3-OMG; however, Wheeler (1989) found no effect of glucose 6-phosphate on glucose uptake into liposomes from reconstituted adipocyte membrane proteins.

Although dexamethasone decreased net uptake of 3-OMG into cells incubated in solution containing zero sugar, there is no evidence of further inhibition of 3-OMG influx on preloading cells exposed to dexamethasone with 2-dGlc or glucose (Table 3).

Preloading the cells with 1 mM or 10 mM 2-dGlc gave a graded *trans*-inhibition of 3-OMG uptake in both control and PMA-treated cells. When cells were preloaded with glucose, no significant PMA-dependent increase above control cells preloaded in similar conditions was observed (Table 4). The effects of pre-incubation with PMA (40 nM) on 3-OMG uptake into cells preincubated in sugar-free solution or with either 2-dGlc or glucose are shown in Table 4. PMA increased 3-OMG (10 mM) uptake above control cells incubated in sugar-free solution ($P < 0.001$) or with 1 mM-2-dGlc ($P < 0.01$).

In summary, PMA increased the component of 3-OMG (10 mM) uptake which can be inhibited by preloading the cells with 2-dGlc or glucose to $1.45 \pm 0.13 \mu\text{mol}/5 \text{ min per } 10^{10}$ cells, above the control value of $0.96 \pm 0.12 \mu\text{mol}/5 \text{ min per } 10^{10}$ cells, i.e. by $52 \pm 8\%$ ($P < 0.01$); dexamethasone abolished *trans*-inhibition of 3-OMG by 2-dGlc or glucose altogether ($0.16 \pm 0.15 \mu\text{mol}/5 \text{ min per } 10^{10}$ cells; $P < 0.01$ versus control).

Effects of preloading with 3-OMG on uptake of 3-OMG and 2-dGlc

Preloading control cells with unlabelled 3-OMG increased the rate of uptake of labelled 3-OMG from solutions containing 5–20 mM labelled 3-OMG (by

approx. 30%, averaged over the entire concentration range; $P < 0.001$) and increased uptake of 20 mM-3-OMG by 41% ($P < 0.001$) (Table 5). This confirms similar effects reported by Whitesell & Regen (1978). In the presence of PMA (40 nM) the range of 3-OMG uptake into cells which had not been preloaded with sugar was significantly increased above control ($P < 0.001$, as also shown in Table 3). Uptake of 3-OMG into PMA-treated cells which had been preloaded with 3-OMG (40 mM) was increased above that seen in PMA-treated cells without preloading, by 30% ($P < 0.01$). However, PMA did not significantly increase 20 mM-3-OMG uptake into cells preloaded with 3-OMG (40 mM) above the rate of exchange influx observed in control cells which were preloaded with 40 mM-3-OMG.

Dexamethasone decreased the rate of 3-OMG uptake into cells which had not been preloaded with sugar, by 15% below that seen in control cells ($P < 0.01$, also shown in Table 3). Preloading the cells with 3-OMG (40 mM) increased the rate of 3-OMG by 69% ($P < 0.001$). This increment in influx caused by preloading in dexamethasone-treated cells ($2.14 \pm 0.21 \mu\text{mol}/5 \text{ min per } 10^{10}$ cells) was significantly larger than the 3-OMG-dependent increment found with control ($1.43 \pm 0.23 \mu\text{mol}/5 \text{ min per } 10^{10}$ cells; $P < 0.05$). The maximum rate of exchange influx into dexamethasone-treated cells did not differ from influx into either control or PMA-treated cells preloaded with 40 mM-3-OMG.

Preloading with 3-OMG also significantly enhanced the rate of 2-dGlc (1 mM) in control or dexamethasone-treated cells. However, 3-OMG (40 mM) did not significantly enhance the rate of uptake of 2-dGlc into PMA-treated cells. The rate of hetero-exchange uptake of 2-dGlc into cells preloaded with 40 mM-3-OMG was not significantly affected by either dexamethasone or PMA. These results indicate that PMA or dexamethasone modulate sugar transport by altering zero-*trans* net uptake, but do not affect exchange uptake.

DISCUSSION

Evidence supporting the view that dexamethasone affects the coupling between 2-dGlc and hexokinase

The following results are consistent with the view that dexamethasone decreases the coupling between 2-dGlc

Table 5. Effects of 3-OMG (40 mM) preloading on 3-OMG uptake at different external concentrations and on 2-dGlc (1 mM) uptake into control cells or cells exposed to PMA (40 nM) or dexamethasone (1 μ M)

Cells were preincubated in unlabelled 3-OMG (40 mM) or sugar-free medium for 2 h at 37 °C with or without PMA or dexamethasone. The cells were then transferred into fresh medium containing the various concentration of either 14 C-labelled 3-OMG or 3 H-labelled 2-dGlc as shown. 3-OMG uptakes were measured over 5 min and 2-dGlc uptakes over 10 min. Results are expressed as means $\pm$ S.E.M. ($n = 8$ observations): *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, test versus control (zero, same row); ††† $P < 0.001$, †† $P < 0.01$, † $P < 0.05$, test versus zero sugar preload; ‡ $P < 0.05$, ns, not significant test versus 40 mM-3-OMG preload control same row.

[3-OMG] (mM)	Preloading concn. of 3-OMG...	Uptake (μ mol/5 min per 10^{10} cells)					
		Control		PMA		Dexamethasone	
		Zero	40 mM	Zero	40 mM	Zero	40 mM
5		1.31 $\pm$ 0.04	1.83 $\pm$ 0.11**	1.67 $\pm$ 0.04***	2.40 $\pm$ 0.30†,‡	1.20 $\pm$ 0.04*	2.04 $\pm$ 0.14††.ns
10		2.82 $\pm$ 0.15	3.35 $\pm$ 0.20*	3.83 $\pm$ 0.20**	4.31 $\pm$ 0.10†,‡	2.17 $\pm$ 0.09**	3.12 $\pm$ 0.11†††.ns
20		3.49 $\pm$ 0.13	4.92 $\pm$ 0.20***	4.43 $\pm$ 0.06***	5.96 $\pm$ 0.58†.ns	3.10 $\pm$ 0.04*	5.24 $\pm$ 0.21†††.ns

[2-dGlc] (mM)	Preloading concn. of 2-dGlc...	Uptake (μ mol/10 min per 10^{10} cells)					
		Control		PMA		Dexamethasone	
		Zero	40 mM	Zero	40 mM	Zero	40 mM
1		1.25 $\pm$ 0.07	1.42 $\pm$ 0.05*	1.44 $\pm$ 0.05*	1.55 $\pm$ 0.09††.ns	1.03 $\pm$ 0.04*	1.46 $\pm$ 0.05††.ns

transport and hexokinase activity at the endofacial surface of the transporter.

(1) The exit of accumulated 2-dGlc from thymocytes is increased by dexamethasone (Fig. 3). Concurrently, dexamethasone slows uptake of 2-dGlc by 30 % (Fig. 1, Table 3).

(2) Dexamethasone decreases steady-state accumulation of 2-dGlc within the cytosol (Table 1).

Hence dexamethasone decreases the asymmetry of 2-dGlc transport across the plasma membrane, which is the converse effect to that produced by PMA, which enhances uptake, decreases exit and increases free sugar accumulation (Naftalin & Rist, 1989). A dexamethasone-dependent decrease in the degree of coupling between hexokinase and sugar movement across the glucose transporter with increased 'slippage' between the transport and phosphorylation processes accounts for both the enhanced rate of 2-dGlc loss from the cytosol and the decreased rate of 2-dGlc uptake and accumulation within the cytosol.

Evidence for absence of change in number of glucose transporters within the plasma membrane of rat thymocytes

The following data indicate that the decreased maximal rates of 2-dGlc and 3-OMG uptake into dexamethasone-treated thymocytes are inconsistent with a decrease in the number of glucose transporters within the cell membrane.

(1) Although dexamethasone decreases the $V_{\max}$ for net uptake of both 2-dGlc and 3-OMG (Figs. 1 and 2), there is no significant dexamethasone-dependent decrease in the rate of exchange uptake of either 2-dGlc (1 mM) or

3-OMG (10 mM) into cells preloaded with 3-OMG (40 mM) (Table 5). Although the decrease in $V_{\max}$ for net uptake of 2-dGlc or 3-OMG could be consistent with a dexamethasone-dependent decrease in the number of glucose carriers within the membrane, this view, at least for the rat thymocyte, can be excluded by the lack of correspondence of dexamethasone-dependent changes in $V_{\max}$ for net uptake with the dexamethasone-dependent changes in the rates of exchange with 3-OMG.

If the numbers of transporters were decreased by dexamethasone, then the rates of exchange flux should also be decreased.

(2) Similarly, although PMA increases the maximal rate of 3-OMG uptake into the thymocytes (Fig. 2), there is no evidence that PMA increases the number of transporters: there is no significant difference between the rate of exchange uptake of 3-OMG (20 mM) or of 2-dGlc into PMA-treated cells preloaded with 3-OMG (40 mM) compared with exchange uptake into control cells or cells exposed to dexamethasone. If PMA increased the number of membrane transporters, then the rate of exchange of uptake of 3-OMG should increase in parallel with the rate of net uptake. Whitesell & Regen (1978) drew similar conclusions from their data.

Evidence indicating that 3-OMG uptake into control, PMA- and dexamethasone-treated tissue is modulated by *trans*-inhibition by cytosolic free sugars

At first sight, modulation of the rate of 3-OMG uptake into thymocytes by PMA and dexamethasone would seem to be inconsistent with the view that transport is modulated by its degree of coupling with hexokinase

activity. The apparent changes in 3-OMG uptake would seem to be more consistent with the conventional view of sugar transport control modulated by the number of transporters present within the cell membrane. However, this view is incompatible with the findings discussed above, so some alternative is required. Whitesell *et al.* (1977) showed that the exchange component of 3-OMG transport into thymocytes was decreased on activation of quiescent cells, and they suggested that activation is due to a form of unmasking of transporter activity. The hypothesis used here to explain modulation of 3-OMG flux in thymocytes is an extension of this view to encompass coupling of transport to hexokinase.

Exchange flux between different sugars can be faster than, slower than, or equal to the rate of zero-*trans* net influx (Naftalin, 1971; Lieb & Stein, 1972). Thus binding of a slowly exchanging sugar, e.g. glucose or 2-dGlc, to the endofacial site of the glucose transporter will slow uptake of a rapidly transported sugar, e.g. 3-OMG, by replacing the rapid net flux component of uptake with the slower hetero-exchange component.

Binding of competitive inhibitors to the *trans*-surface of the transporter generates quasi-non-competitive inhibition of influx, i.e. a decrease in $V_{\max}$ of uptake, without change in the K_m (Deves & Krupka, 1978; Krupka & Deves, 1979; Basketter & Widdas, 1978). If free glucose or 2-dGlc is accumulated within the cytosol before measurement of 3-OMG uptake, either sugar will decrease the $V_{\max}$ for 3-OMG uptake by *trans*-retardation of exchange uptake. The endofacial activity of hexokinase will alter the proportion of transporter sites liganded to glucose from cytosolic sources, i.e. activation by PMA will decrease the proportion of endofacial sites liganded to glucose and consequently increase the rate of 3-OMG uptake, whereas dexamethasone, which decreases surface activity of hexokinase, will increase the proportion of endofacial transporter sites liganded to glucose and so decrease the $V_{\max}$ of 3-OMG uptake.

Similarly, starvation decreases cytosolic free sugar and hence will increase the $V_{\max}$ of 3-OMG uptake by increasing the net flux and decreasing hetero-exchange components of influx. As starvation and PMA both decrease the proportion of endofacial sites liganded to Glc, starvation will decrease the augmentation of 3-OMG influx by PMA. However, starvation will not prevent the PMA-dependent increase in 2-dGlc uptake, because 2-dGlc uptake is mainly controlled by the rate at which it is removed from the endofacial surface by the endofacial hexokinase activity.

Exchange flux of labelled 3-OMG with high concentrations of unlabelled cytosolic 3-OMG will not be affected by changes in endofacial activity of hexokinase, as the cytosolic 3-OMG will displace a high proportion of the retarding glucose from the endofacial sites of the transporter. As dexamethasone decreases endofacial hexokinase activity, the proportion of endofacial sites liganded to glucose will be increased; consequently, loading the dexamethasone-pretreated cells with 3-OMG will unmask a larger exchange flux.

The experiments in Tables 3, 4 and 5 and also Fig. 4 were designed to test the view that 3-OMG uptake is modulated by the endofacial activity of slowly exchanging sugars.

The following results are consistent with this view.

(a) In control and PMA-treated cells, preloading cells for 60 min with graded concentrations of 2-dGlc inhibits

3-OMG (10 mM) uptake by 32% (control) and 38% (PMA) (Table 4). Preloading with glucose (10 mM) inhibits 3-OMG uptake to a similar extent (Table 4).

(b) PMA increases net influx of 3-OMG without affecting the maximum exchange flux of 3-OMG (Table 5).

(c) Dexamethasone decreases net influx of 3-OMG without affecting exchange flux of 3-OMG (Table 5).

(d) PMA increases net influx of 2-dGlc, and preloading PMA-treated cells with 3-OMG has no effect on 2-dGlc influx (Table 5).

(e) Dexamethasone decreases net influx of 2-dGlc (Table 5, Fig. 1) and significantly enhances the effect of 3-OMG on 2-dGlc uptake into dexamethasone-treated cells.

Evidence indicating that cytosolic free glucose inhibits uptake of 3-OMG

(1) Displacement of the free sugar from the endofacial surface by the competitive action of cytosolic 3-OMG is the only satisfactory explanation for the parity of the maximal exchange fluxes in control, PMA and dexamethasone-treated cells, i.e. the rates of uptake of 3-OMG (20 mM) into control, PMA- or dexamethasone-treated cells preloaded with 40 mM-3-OMG do not differ significantly (Table 5).

(2) Influx of 3-OMG is decreased in both control and dexamethasone-treated tissue by preloading with unlabelled 2-dGlc or glucose (Table 3).

(3) In PMA-treated cells the rate of uptake of 3-OMG is not decreased below that seen into the control cells until the cells are preloaded with 10 mM-2-dGlc or -glucose (Table 4). This shows that, even with significant amounts of unlabelled 2-dGlc present within the cytosol of PMA-treated cells (i.e. 2–5 mM; see Table 1, and Naftalin & Rist, 1989), uptake of 3-OMG is still higher than in control cells which have not been preloaded with sugar. This finding is consistent with the view that decreased binding of 2-dGlc to the endofacial surface as a result of the PMA-dependent increase in endofacial hexokinase activity raises influx of 3-OMG.

(4) Preloading with 3-OMG stimulates uptake of 3-OMG in dexamethasone-treated or control cells to a greater extent than into PMA-treated cells.

(5) Starvation for more than 3 h enhances 3-OMG uptake into control cells and prevents the PMA-dependent enhancement of 3-OMG uptake. These effects can be prevented by incubation with 2-dGlc (Fig. 4).

These results imply that thymocytes maintain a significant intracellular concentration of free glucose after isolation and incubation in glucose-free medium.

Although it is apparent that modulation of sugar transport in rat thymocytes is unlikely to be accompanied by a large change in the numbers of plasma-membrane glucose carriers, this does not necessarily apply to transport regulation in other cell systems, e.g. adipocytes, where much larger changes in regulated influx are found (Simpson & Cushman, 1986).

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