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Glucose transport by cultured human fibroblasts: regulation by phorbol esters and insulin

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The regulation of 3-*O*-methyl-D-glucose (OMG) uptake by insulin and phorbol esters was studied in cultured human skin fibroblasts. Insulin rapidly stimulated OMG uptake through a mechanism independent of new protein synthesis. Maximal insulin effect was reached in 30 min and remained constant up to 12 h. The protein kinase C activators 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) and phorbol 12,13-dibutyrate (PdBU) promoted an initial rapid stimulation followed by a secondary long-term rise of OMG influx. This latter effect of phorbol esters on OMG influx began after 1 h, reached a maximum in 12–15 h, and was prevented by the simultaneous addition of protein synthesis inhibitors, suggesting that phorbol esters increased the synthesis of new glucose transporters. In accord with this interpretation, phorbol esters, but not insulin, increased mRNA levels for two distinct glucose transporters (GLUT1 and GLUT3) in human fibroblasts. Both the rapid and the long-term effects of phorbol esters on OMG influx were dose-dependent and half-maximal stimulations occurred at 15 nM for both PdBU and TPA. Kinetic analysis of OMG uptake indicated that both effects of phorbol esters were associated with an increase in the V_{max} of the transport process, with no significant changes of the K_m (4–6 mM). These results suggest that, in human fibroblasts, phorbol esters, unlike insulin, produce a long-term stimulation of OMG uptake, which is dependent upon protein synthesis and is associated with increased levels of GLUT1 and GLUT3 mRNA.

Introduction

Glucose enters human fibroblasts by facilitated diffusion down its chemical gradient [1]. The rate at which this transport process takes place is stimulated by insulin [2,3]. Insulin initiates its action by interacting with specific receptors on the plasma membrane of target cells. These receptors are heterotetramers composed of two α and two β subunits linked by disulfide bonds. The α subunit is extracellular and binds insulin whereas the β subunit spans the plasma membrane and has an intracellular tyrosine kinase domain [4,5]. Insulin binding to the α subunit of the receptor stimulates β subunit autophosphorylation and kinase activity. In fibroblasts derived from patients with inherited insulin-resistant syndromes, defective insulin receptor autophosphorylation and kinase activity are associated with defective insulin stimulation of sugar uptake [6–8]. By contrast, human fibroblasts with a constitutive activation of the insulin receptor kinase have increased and insulin-insensitive glucose transport [9,10], indicating that the kinase activity of the insulin receptor plays an essential role in the stimulation of glucose transport.

Several candidates have been proposed for the next step in the transduction of the insulin signal. Protein kinase C has been involved in insulin stimulation of glucose transport, since phorbol esters which activate protein kinase C also stimulate sugar transport by many cell types including adipocytes [11], BC3H-1 myocytes [12] and mouse skeletal muscle [13]. In addition, insulin increases intracellular levels of diacylglycerol, the natural activator of protein kinase C, in some cell types [14,15]. However, other serine/threonine kinases may be activated by lipid mediators and stimulation of glucose transport has been observed with diacylglycerols which do not stimulate protein kinase C [16].

Abbreviations: BSA, bovine serum albumin; DV, Dulbecco-Vogt (medium); EBSS, Earle's balanced salt solution; OMG, 3-*O*-methyl-D-glucose; PdBU: phorbol 12,13-dibutyrate; TPA, 12-*O*-tetradecanoyl phorbol 13-acetate.

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Like insulin, phorbol esters rapidly stimulate sugar transport in human fibroblasts [17]. However, the long-term effects of insulin and phorbol ester on glucose transport have not been characterized in cultured cells and it is unknown whether phorbol esters modulate glucose transporter mRNA levels in human fibroblasts. In this study, we compare short and long-term effects of insulin and phorbol esters on OMG transport by human fibroblasts. Both compounds rapidly enhanced OMG influx, but only phorbol esters caused a further increase of OMG uptake sustained over time (long-term stimulation). This latter effect of phorbol esters was associated with increased mRNA levels for two glucose transporters, GLUT1 and GLUT3 (for nomenclature, see Ref. 18).

Materials and Methods

Materials. Fetal bovine serum (FBS) and growth media were from GIBCO. 3-*O*-[^{3}H]Methyl-D-glucose (79 Ci/mmol) was from New England Nuclear; [^{32}P]dCTP was from Amersham. Sigma and Aldrich were the sources of phorbol esters, cytochalasin B, protein synthesis inhibitors, and other chemicals.

Cell culture. Human fibroblasts were derived from adult skin biopsies and propagated in Dulbecco-Vogt (DV) medium supplemented with 15% fetal bovine serum. For uptake assays, fibroblasts ($4 \cdot 10^4$ cells) were seeded in 24-well plates (Costar) and used after 8–12 days. The culture medium was renewed every 72 h and 48 h before the experiment.

Glucose transport. Confluent monolayers were washed twice and incubated at 37°C in Earle's balanced salt solution (EBSS) containing D-glucose (25 mM) and 0.5% bovine serum albumin (BSA, RIA grade) with the indicated additions. Insulin, when added was used at 200 nM, a concentration which maximally stimulates glucose transport by human fibroblasts [9]. At the indicated time, cells were washed three times with glucose-free EBSS and 3-*O*-[^{3}H]methyl-D-glucose (OMG, 1 mM) influx was measured for 10–20 s, a time-frame which allows the measurement of initial rates of entry of this sugar in human fibroblasts [1]. The uptake assay was stopped by three rapid washes with ice-cold 0.1 M $MgCl_2$. Intracellular radioactivity was extracted with 0.5 ml of ethanol which were then added to 2.5 ml of scintillation fluid (EcoScint, National Diagnostic) and counted in a liquid scintillation spectrometer (Beckman LS 7500). Protein content was measured in each well by a modified Lowry procedure [19] and intracellular fluid volume was estimated from the equilibrium distribution of OMG [20]. Previous studies indicated that cell water estimated with this method in human fibroblasts is identical to that obtained from the equilibrium distribution of inulin and urea [1]. In different experiments and conditions, intracellular water ranged between 6.1 and 8.0 μl/ mg of cell protein, with a mean value of 7.05 ± 0.71 μl/mg of cell protein.

Calculations. OMG uptake was normalized to cell protein and cell water and expressed as nmol/ml cell water per s. The analysis of initial-velocity kinetic curves was performed using a BASIC program applying Marquardt's algorithm for least-squares estimation of nonlinear parameters [21]. The equations used were:

$$v = \frac{V_{max} \cdot [S]}{K_m + [S]} \quad (1)$$

where [S] is the concentration of substrate, V_{max} is the maximal velocity and K_m is the Michaelis-Menten constant. This equation was used on data corrected for non-saturable OMG uptake, measured in the presence of 10 μM cytochalasin B [1].

The analysis of dose-reponse curves was performed using the stimulation above control values (difference between stimulated and control values) by means of the following equation:

$$\text{Stimulation} = \frac{E_{max} \cdot [\text{AGONIST}]}{ED_{50} + [\text{AGONIST}]} \quad (2)$$

where E_{max} is the maximal effect and the ED_{50} the concentration of agonist at which half-maximal response in observed [22].

Parameters obtained by nonlinear regression analysis are shown in the text with 95% confidence intervals. Other data are reported as means $\pm$ S.D. of at least three determinations.

RNA analysis. Fibroblasts were grown to confluence in 150 cm^2 flasks and incubated for the indicated time in the presence of insulin or phorbol esters. At the end of the incubation cells were washed with sterile ice-cold saline and RNA was extracted by guanidinium thiocyanate [23]. Total RNA (10–20 μg/lane) was separated by formaldehyde-agarose gel electrophoresis, blotted to nylon (ZetaProbe, Bio-Rad), and hybridized at 65°C to ^{32}P-labeled cDNAs coding for GLUT1 [24], GLUT3 [25], and GLUT4 [26] glucose transporters. The hybridization solution contained 7% sodium dodecylsulfate, 1% bovine serum albumin, 10% poly(ethylene glycol), 0.25 M NaCl, 1 mM EDTA, and 0.16 M sodium phosphate. Blots were washed at 65°C in 0.5 × SSC and exposed for 18–48 h at −70°C. Blots were then stripped and re-hybridized to β-actin cDNA. Autoradiograms were analyzed by two-dimensional laser densitometry and intensity of the glucose transporter band was normalized to the intensity of the actin signal before comparison.

Results and Discussion

Time-course for insulin and TPA stimulation of glucose transport

The effect of phorbol esters (1 μM) on OMG influx in human fibroblasts is reported in Table I. Phorbol esters which activate protein kinase C stimulated OMG influx in human fibroblasts. The inactive phorbol ester, phorbol 13-monoacetate had no significant effect on OMG influx. Addition of diacylglycerol, a natural activator of protein kinase C, stimulated sugar influx to a lesser extent, perhaps because of rapid inactivation. In different experiments and in different fibroblast strains, phorbol esters stimulated OMG influx between 50 and 200% above basal after 30 min of incubation.

The time course of TPA and insulin stimulation of 3-*O*-methyl-D-glucose (OMG) transport is shown in Fig. 1. To avoid the interference of serum factors, human fibroblasts, grown to confluence in the presence of serum, were washed and incubated either in serum-free Earle's balanced salt solution (EBSS, panel A) or in serum-free Dulbecco-Vogt medium (DV, panel B), in the presence of either insulin or TPA. Incubation in the absence of serum caused a decrease in OMG uptake that reached a minimum after 3 h. After this time, basal OMG uptake remained constant up to 12 (panel A) or 48 h (panel B). Insulin, added at the beginning of the incubation, prevented this decline in sugar transport and stimulated OMG influx above the value observed in serum-treated cells. Insulin stimulation of OMG uptake was rapid. Significant increases in OMG influx occurred after 5 min of incubation (data not shown), reached a maximum in about 30 min, remained constant for 12 h and declined thereafter. However, even after 48 h of incubation, OMG uptake was still significantly higher in cells incubated in the presence of insulin than in cells incubated in DV medium alone (Fig. 1B). The increase in OMG influx

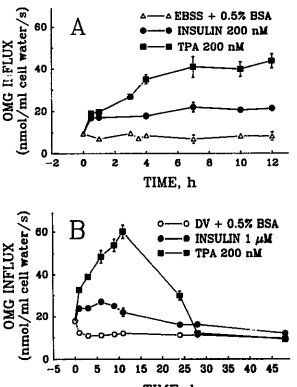

Fig. 1. Time course of the effect of insulin (200 nM, 1.2 μg/ml) and 12-*O*-tetradecanoyl phorbol 13-acetate (TPA, 200 nM) on 3-*O*-methyl-D-glucose (OMG) uptake by cultured human fibroblasts. Human fibroblasts were grown to confluence in Dulbecco-Vogt (DV) medium supplemented with 15% fetal bovine serum (FBS). (A) Cultures were washed and incubated at 37°C in Earle's balanced salt solution (EBSS) supplemented with bovine serum albumin 0.5% in the presence of either insulin or TPA. At the indicated times, cells were washed three times with glucose-free EBSS and OMG (1 mM) influx was measured for 15 s. (B) Confluent cultures of human fibroblasts were washed and incubated at 37°C in Dulbecco-Vogt medium supplemented with 0.5% BSA, in the presence of either insulin (200 nM) or TPA (200 nM). OMG (1 mM) uptake was then measured for 10 s and normalized to intracellular water. Intracellular water did not differ among points at the same time interval. Each point is the mean of triplicates ±S.D. Each experiment was repeated twice with similar results.

TABLE I

Effect of phorbol esters and diacylglycerol on 3-O-methyl-D-glucose (OMG) influx in cultured human fibroblasts

Confluent cultures were incubated for 30 min in the presence of the indicated phorbol ester (1 μM) or diacylglycerol (20 μM). The influx of OMG (1 mM) was then measured for 20 s. Data are expressed as mean ± S.D. of at least three determinations. TPA 12-*O*-tetradecanoyl phorbol 13-acetate. * $P < 0.05$, ** $P < 0.01$ versus control, using variance analysis.

	OMG uptake (nmol ml^{-1} s^{-1})	Effect (%)
Control (EBSS)	13.81 ± 1.90	–
TPA	33.31 ± 1.00	+ 141 **
Phorbol 12,13-dibutyrate	32.59 ± 0.41	+ 136 **
Phorbol 13-monoacetate	15.49 ± 0.42	+ 12
Diacylglycerol	18.48 ± 1.33	+ 34 *

promoted by insulin was not observed when the uptake measurement was performed in the presence of cytochalasin B (20 μM). Cytochalasin B-insensitive OMG influx was 1.9 ± 0.5 nmol/ml of cell water per s in cells incubated for 1 h in EBSS and 1.7 ± 0.4 nmol/ml cell water per s in cells incubated for the same time in the presence of insulin.

TPA mimicked the rapid stimulation of OMG influx caused by insulin (Fig. 1). Additionally, TPA promoted a second increase in sugar uptake, that was sustained for 12 h (long-term stimulation). After this period, OMG transport in TPA-stimulated cells slowly declined and returned to the basal value in about 30 h (Fig. 1B). This decrease was probably due to a down regulation of protein kinase C activity which is observed in human fibroblasts [27] and other cell types

[13,28]. TPA did not affect cytochalasin B-insensitive OMG uptake, which remained 1.9 ± 0.3 nmol/ml cell water per s in cells incubated for 1 h or 6 h with the phorbol ester. In several experiments, even after 1 h of incubation, TPA was significantly more effective than insulin in stimulating OMG uptake, whereas shorter times of incubation (up to 15–30 min) resulted in similar stimulations by the two agonists.

These results indicate that phorbol esters, in addition to an acute effect, have a long-term effect on OMG uptake which is not shared with insulin. The long-term effect of phorbol esters on OMG uptake was evident even at short times of incubation (1 h). This additional long-term effect of phorbol esters on OMG uptake may explain the higher stimulation of glucose transport obtained by TPA as compared to insulin in human fibroblasts as well as in other cultured cells [29]. By contrast, in adipose and muscle cells in primary culture, insulin is 3–5-fold more powerful than phorbol esters in stimulating glucose transport (for review, see Ref. 29). This dramatic difference is probably attributable to the different glucose transporters expressed by these cells. GLUT1 is the predominant isoform in human fibroblasts [30], whereas GLUT4 represents the major glucose transporter of both adipocytes and muscle cells [26].

Effect of protein synthesis inhibitors on insulin and TPA stimulation of glucose transport

The effect of protein synthesis inhibitors on insulin and TPA stimulation of sugar transport is shown in Fig. 2. Fibroblasts were incubated for 30 min (panel A) or 8 h (panel B) in EBSS containing insulin or TPA and protein synthesis inhibitors. 30 min were selected to reduce the contribution of the long-term effect of TPA on the stimulation of OMG uptake. Neither cycloheximide nor actinomycin D prevented the rapid stimulation of OMG uptake by insulin or TPA (Fig. 2A). Cycloheximide (18 μM) alone rapidly stimulated sugar transport, but its stimulation was additive to that produced by insulin and TPA (Fig. 2A). Actinomycin D (5 μM) significantly increased OMG uptake after 8 h, but not after 30 min of incubation. After 8 h of incubation, insulin stimulation of OMG uptake was not impaired by protein synthesis inhibitors (Fig. 2B). By contrast, both cycloheximide and actinomycin D abolished the portion of TPA stimulation of OMG uptake above that observed after 30 min of incubation (Fig. 2A). These results indicated that the rapid stimulation of OMG uptake by both insulin and TPA was independent of protein synthesis, whereas the long-term effect of TPA depended upon new protein synthesis.

Cycloheximide rapidly stimulated sugar transport (Fig. 2A). This stimulation (30 to 100% above basal OMG uptake in different experiments) was dose-dependent and half maximal stimulation was obtained

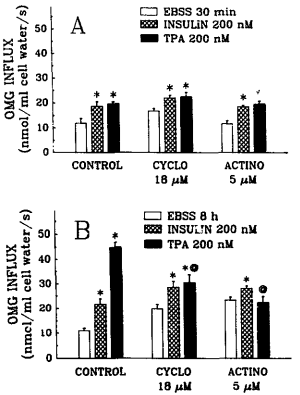

Fig. 2. Effect of cycloheximide and actinomycin D on insulin and TPA stimulation of OMG transport by human fibroblasts. Confluent cultures of human fibroblasts were incubated for 30 min (panel A) or 8 h (panel B) at 37°C in EBSS supplemented with 0.5% BSA (control) in the absence or in the presence of insulin (200 nM), TPA (200 nM), cycloheximide (cyclo, 18 μM) or actinomycin D (actino, 5 μM). OMG (1 mM) transport was then measured for 10 s. Each point represents the mean of triplicates $\pm$S.D. The experiment was repeated twice with similar results. Addition of cycloheximide for 30 min (panel A) and of cycloheximide or actinomycin D for 8 h (panel B) significantly ($P < 0.01$) increased OMG uptake in the absence of added insulin or TPA. * $P < 0.01$ vs. paired EBSS; # $P < 0.01$ vs. TPA alone, using analysis of variance.

with 1.1 ± 0.2 μM of cycloheximide, a value close to that producing half-maximal inhibition of protein synthesis in human fibroblasts (1.5 ± 0.3 μM). Other investigators found a stimulation of glucose transport by cycloheximide in BC3H-1 myocytes [31] and in adipocytes [32]. Since cycloheximide also stimulates protein kinase C activity [31,33], it is possible that this protein synthesis inhibitor stimulates sugar transport by interacting with protein kinase C [31]. In addition, cycloheximide may also prevent protein kinase C down regulation and degradation in human fibroblasts [27]. Our results do not rule out these possibilities, but show that the stimulation of OMG uptake by phorbol esters and cycloheximide is additive (Fig. 2) and that another protein synthesis inhibitor, actinomycin D, also stimulates sugar transport when the incubation is continued for enough time to observe its action (Fig. 2B). It is possible that the stimulation of glucose transport by cycloheximide is related to its action on protein synthesis. In support of this hypothesis, another protein syn-

thesis inhibitor, anisomycin, produced a similar stimulation of glucose transport (not shown).

Effect of insulin and phorbol esters on glucose transporter mRNA

Three different types of glucose transporter mRNA have been identified in human fibroblasts (GLUT1, GLUT3 and GLUT4). GLUT1 and GLUT3 representing the major classes [30]. The effect of insulin and TPA on GLUT1 and GLUT3 mRNA levels is shown in Fig. 3. Human fibroblasts were incubated for 6 and 48 h in culture medium in the absence or in the presence of insulin (1 μM) or TPA (200 nM). After 6 h of incubation, TPA, but not insulin, increased both GLUT1 and GLUT3 mRNA levels. Quantification of mRNA levels by laser densitometry (see Methods) in three separate experiments indicated that 6 h of incubation with TPA increased GLUT1 and GLUT3 mRNA levels between 2.5- and 3.5-times (mean 3.1 ± 0.5-fold). This increase was comparable to the increase in OMG uptake observed in cells incubated for 6–10 h with TPA (Fig. 1). After 48 h of incubation with TPA, GLUT1 and GLUT3 mRNA levels ranged between 0.9- and 1.2-times those observed in cells incubated in plain medium in three separate experiments (Fig. 3).

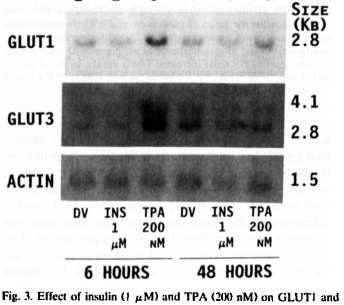

Fig. 3. Effect of insulin (1 μM) and TPA (200 nM) on GLUT1 and GLUT3 mRNA in human fibroblasts. Confluent cells were incubated in DV medium supplemented with 0.5% bovine serum albumin in the absence (DV, lanes 1 and 4) or in the presence of insulin (lanes 2 and 5) or TPA (lanes 3 and 6). After 6 h (lanes 1–3) and 48 h (lanes 4–6), total RNA was extracted by guanidinium thiocyanate, separated by gel electrophoresis (20 μg/lane), blotted to nylon, and hybridized to [^{32}P]cDNA encoding the GLUT1 and the GLUT3 glucose transporters. The hybridization signal was detected by autoradiography. Blots were then re-hybridized to actin cDNA for normalization. Band size was calculated by comparison with the migration of an RNA ladder (BRL). The experiment was repeated three times with similar results.

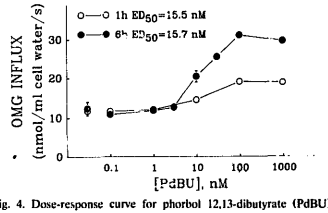

Fig. 4. Dose-response curve for phorbol 12,13-dibutyrate (PdBU) stimulation of OMG transport in human fibroblasts. Confluent cultures of human fibroblasts were incubated for 1 h or 6 h in EBSS containing 0.5% BSA in the presence of the indicated concentrations of PdBU. OMG (1 mM) uptake was then measured for 10 s. Each point represents the mean of triplicates ±S.D. PdBU-stimulated OMG uptake (difference between uptake measured in the presence and in the absence of PdBU) was fitted to Eqn. 2 (see Methods) to obtain ED_{50} values.

This latter finding correlated well with the return to basal levels of glucose transport in cells treated with TPA for 48 h (Fig. 1). Neither insulin nor TPA affected the low levels of GLUT4 mRNA in human fibroblasts (not shown).

An effect of TPA on GLUT1 mRNA levels and glucose transport has been observed in mouse 3T3 fibroblasts [34]. In the case of the recently discovered GLUT3 transporter [25], stimulation of mRNA levels by phorbol esters in human fibroblasts represents the first type of regulation described.

Insulin, unlike TPA, was unable to produce a sustained long-term stimulation of glucose transport (Fig. 1) and, accordingly, did not increase glucose transporter mRNA (Fig. 3). These data contrast with those of Kosaki and collaborators [35] who found that insulin increased GLUT1 mRNA levels of 60% above basal in human fibroblasts. Glucose transport and GLUT3 mRNA were not investigated in the reported study [35]. The reasons for this discrepancy are unclear at the moment. Methodological differences, such as a the prolonged serum starvation before insulin addition [35], may have contributed to produce or to enhance insulin effect in the previous study.

Dose-response curve for phorbol ester stimulation of glucose transport

Both the rapid and the long-term effects of phorbol esters on OMG influx were dose-dependent. Fig. 4 shows dose-response curves for PdBU stimulation of OMG influx after 1 h or 6 h of incubation. PdBU was used in this quantitative experiment to avoid problems of non-specific binding to lipid components observed with TPA. The maximal stimulation of OMG influx in human fibroblasts was obtained at 100 nM of PdBU

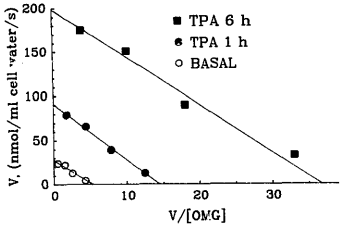

Fig. 5. Effect of TPA (200 nM) on the kinetic constants for OMG transport by human fibroblasts. Confluent monolayers were incubated for 1 and 6 h at 37°C in EBSS containing 0.5% BSA in the absence or in the presence of TPA (200 nM). OMG (1–45 mM) uptake was then measured for 10 s in the absence or in the presence of cytochalasin B (10 μM) added to the cells 3 min before measuring uptake. Cytochalasin B-insensitive OMG influx was subtracted before plotting according to an Eadie-Hofstee graphical representation. Each point is the mean of triplicates. Data were analyzed by nonlinear regression according to a Michaelis-Menten equation (Eqn. 1 in Methods) and lines represent the best fit obtained to each set of data.

and the half-maximal effect was observed at about 15 nM. With TPA, half-maximal stimulation of OMG transport was observed at 17.9 nM (not shown). These values are in the range of the dissociation constant (K_d) for high-affinity binding of phorbol esters to cultured cells [36].

Only those phorbol esters which activate protein kinase C stimulated OMG influx (Table I). Furthermore, half-maximal stimulation was observed at concentrations (10–20 nM) close to the affinity constant for phorbol ester binding to protein kinase C [35]. These results indicate that phorbol esters stimulate OMG uptake in cultured human fibroblasts by activating protein kinase C.

Effect of phorbol esters on the kinetic constants for OMG uptake

The effect of TPA (200 nM) on the kinetic of OMG (1–45 mM) influx is shown in Fig. 5. OMG uptake occurred through a saturable route, inhibitable by cytochalasin B (10 μM), and an unsaturable route, formally indistinguishable from diffusion. TPA did not affect the unsaturable component of OMG influx and the K_d (diffusion constant) was 2.45 s^{-1} in fibroblasts exposed or not to TPA. The unsaturable component was subtracted from total OMG influx to obtain the saturable (cytochalasin B-sensitive) uptake that is reported in Fig. 5 according to an Eadie-Hofstee graphical representation. A 1-h incubation with 200 nM TPA increased the V_{max} for OMG uptake from 27.8 ± 2.0 to 91.1 ± 2.5 nmol/ml per s, without affecting the apparent K_m (from 5.3 ± 1.3 to 6.3 ± 0.6 mM). A more marked increase in the V_{max} value (up to 198.3 ± 8.6 nmol/ml cell water per s, with no change in the apparent K_m value (5.4 ± 0.8 mM), was observed in cells incubated 6 h with TPA.

TPA, like insulin [2], increased the V_{max} for glucose transport, without affecting the K_m. This suggests that both the rapid and long-term effect of phorbol esters are due to an increased number of glucose transporters on the plasma membrane or to increased intrinsic activity of preexisting glucose transporters. Since the long-term effect of phorbol esters can be prevented by the addition of protein synthesis inhibitors (Fig. 2) and is associated with increased glucose-transporter mRNA (Fig. 3), it is more likely that at least this latter effect of TPA is due to an increased number of active transporters on the plasma membrane.

Many studies have focused on the acute effects of insulin and phorbol esters on glucose transport in a variety of cells [11–13]. In this report, we evaluate the long-term effects of insulin and phorbol esters on glucose transport. Although both insulin and phorbol esters produced a rapid stimulation of OMG uptake, phorbol esters also promoted a secondary, long-term stimulation of glucose transport which was associated with increased levels of GLUT1 and GLUT3 mRNA. This latter finding, together with the sensitivity of this secondary increase in glucose transport to protein synthesis inhibitors, suggested that phorbol esters stimulated the synthesis of new transporter proteins. This additional long-term effect of phorbol esters may explain why in cultured cells TPA seems more effective in stimulating glucose transport than insulin [29]. By contrast, in the skeletal muscle, phorbol esters and intracellular lipid mediators generated by phospholipase C do not activate glucose transport to the extent of insulin [37]. This discrepancy between a cultured cellular model, which expresses mainly the GLUT1 transporter isoform, and the intact muscle, which expresses mainly GLUT4 transporters, may be due to a differential sensitivity to activation by protein kinase C of GLUT1 and GLUT4 transporters.

A further difference between human fibroblasts and the skeletal muscle is in the differential sensitivity to long term insulin incubation. Prolonged incubation with insulin stimulates a protein synthesis-dependent increase in glucose transport by skeletal muscle [38]. By contrast, human fibroblasts failed to increase their glucose transport after a prolonged incubation in the presence of insulin (Fig. 1). Studies in other cell types suggest that differential availability of functional insulin receptors might explain this discrepancy. In fact, in 3T3 F442A murine cells, insulin could stimulate the accumulation of GLUT1 mRNA levels only after differentiation into adipocytes and expression of a high

number of insulin receptors [39]. GLUT1 mRNA accumulation became insulin-sensitive when 3T3 F442A fibroblasts were transfected with normal insulin receptors [39]. Human fibroblasts express a relatively low number of insulin receptors (1000/cell [9]) as compared to muscle cells. However, other possible explanations, such as the differential availability of *trans*-factors capable of modulating gene expression, could explain the different long-term regulation of glucose transport in fibroblasts as compared to differentiated cells. The discordant effects of insulin and TPA on the long-term regulation of glucose uptake in human fibroblasts may be due to the fact that these compounds use different signaling mechanisms or that TPA activates an additional subtype of protein kinase C which is not activated by insulin. Studies of glucose transport and protein kinase C activity in the skeletal muscle support the hypothesis that insulin stimulates glucose transport independent of protein kinase C activation [37]. However similar studies have not been conducted in human fibroblasts, and further studies are required to evaluate the involvement of protein kinase C in insulin action in these cells.

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