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Regulation of glucose transport activity and expression of glucose transporter mRNA by serum, growth factors and phorbol ester in quiescent mouse fibroblasts

Takayuki Kitagawa, Mariko Tanaka and Yuzuru Akamatsu

Department of Chemistry, National Institute of Health, Shinagawa-ku, Tokyo 141 (Japan)

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We have investigated the effects of growth factors such as serum, platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) on glucose transport activity in quiescent mouse Swiss 3T3 cells. DNA synthesis was synchronously induced by either calf serum, or platelet-poor plasma in combination with PDGF or FGF. Early stimulation of glucose transport in the quiescent cells was also caused by serum, or by either PDGF or FGF. The time courses for the stimulation of transport were identical for serum, PDGF and FGF, and the stimulated uptake in each case was associated with a 5–6-fold increase in V_{max}. There were no detectable changes in apparent K_m. Expression of glucose transporter mRNA was also enhanced by these growth factors. By contrast, EGF, insulin and platelet-poor plasma had little effect on glucose transport and transporter-gene expression, although uridine uptake was enhanced by all of these growth factors. These results suggest that cell cycle-dependent stimulation of glucose transport and expression of the transporter mRNA are regulated by a specific class of growth factors such as PDGF and FGF. The tumor promoter phorbol 12-myristate 13-acetate (PMA) also stimulated glucose transport and expression of transporter mRNA in quiescent 3T3 cells. These stimulations were absent in PMA-pretreated cells. However, serum, PDGF and FGF were able to stimulate glucose transport as well as expression of the transporter mRNA in PMA-pretreated cells, suggesting that there are at least two independent pathways for regulating glucose transport and glucose transporter mRNA level in quiescent fibroblasts.

Introduction

Cultured cells arrested in the G_0 phase undergo synchronous progression through the cell cycle upon the addition of whole serum or a combination of growth promoting factors, such as PDGF, FGF, insulin [1]. In recent years, evidence has accumulated that these growth factors can be separated into two distinct classes with respect to their potentiating action on the cell cycle [2,3]: one class, including PDGF and FGF, renders quiescent cells competent to synthesize DNA; the second class, including somatomedin C (IGF-I) and insulin, potentiate the mitogenic effects of the competence factors.

Stimulation of glucose transport is one of the early events that occur prior to the reinitiation of DNA synthesis in quiescent cells [1,3,4]. Transport is also activated by various growth-promoting agents such as serum [5–7], insulin [8–10], EGF [11,12], phorbol esters [13,14], FGF [15] and transforming growth factor β [16]. Activity is also modulated by transformation [17,18], differentiation [10,19] and glucose starvation [20]. However, the molecular mechanism for these changes in glucose transport remains unknown. Since glucose enters mammalian cells by a carrier-mediated diffusion through one or more specific glucose transporters in the plasma membrane, growth factors and transforming gene products could affect glucose transport activity either by modulating the transport activity directly or by modulating the localization and biosynthesis of the transporter [18–21]. Recently, the cDNA encoding a hexose transporter homologous to the hu-

Abbreviations: PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; EGF, epidermal growth factor; PMA, phorbol 12-myristate 13-acetate; PDBu, phorbol 12,13-dibutyrate; OAG, 1-oleoyl-2-acetylglycerol; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; FCS, fetal calf serum; CH, cycloheximide; RBC, red blood cell.

Correspondence: T. Kitagawa, Department of Chemistry, National Institute of Health, Shinagawa-ku, Tokyo 141, Japan.

man RBC transporter was cloned from rat brain [22] and human hepatoma cells [23].

In the present study, we attempted to determine what kinds of growth factors are the most effective in stimulating glucose transport activity in quiescent Swiss 3T3 cells during the course of the transition from the G_0-arrested state to the proliferative state. We found that glucose transport and the content of transporter mRNA are increased by growth factors which exhibit competence activity, such as PDGF, FGF and PMA. We also suggest that there are at least two independent pathways for stimulation of glucose transport in quiescent cells. A preliminary report of this work in an abstract form has been published elsewhere [24].

Materials and Methods

Chemicals. [*Me*-^{3}H]Thymidine (25 Ci/mmol), 2-deoxy[1-^{3}H]glucose (30 Ci/mmol), 3-*O*-methyl[1-^{3}H]glucose (5.9 Ci/mmol), [5-^{3}H]uridine (29 Ci/mmol) and [α-^{32}P]dCTP (3000 Ci/mmol) were obtained from the Radiochemical Center, Amersham, U.K. Other chemicals and inhibitors were obtained from Sigma, St. Louis, MO.

Growth factors. Fetal calf serum and calf serum, and platelet-poor plasma prepared from fresh bovine serum were purchased from Flow Laboratories, Osaka, Japan and Biomedical Technologies, Inc., Cambridge, MA, respectively. Highly purified human PDGF (HPLC grade) was obtained from Collaborative Research Inc., Lexington, MA. Bovine brain FGF and mouse submaxillary gland EGF were obtained from Toyobo Co., Osaka, Japan. Bovine insulin and phorbol 12-myristate 13-acetate (PMA) were obtained from Sigma and LC Service Co., MA, respectively.

Cell culture. Swiss 3T3 cells were maintained in DMEM supplemented with 10% fetal calf serum (FCS), penicillin (100 units/ml) and streptomycin (100 μg/ml) at 37°C in a humidified atmosphere of 5% CO_2, as described previously [25]. At 6–8-week intervals, fresh cultures were initiated.

Assay of DNA synthesis. 3T3 cells were grown in 35-mm plastic dishes in 2 ml of DMEM containing 10% FCS for 5–7 days. Confluent cultures were then incubated for 18–24 h in medium containing 0.5% FCS. The quiescent monolayers were then incubated for a further 24 h in 1 or 2 ml of DMEM containing [^{3}H]thymidine (0.2 μCi/ml, 1 μM) and various concentrations of growth factors. Following incubation at 37°C, the cells were washed with ice-cold PBS and fixed with cold 5% trichloroacetic acid. The acid-precipitable fraction was solubilized in 0.5 M NaOH, neutralized with HCl, and then the radioactivity was measured with a liquid scintillation counter. The quiescent cells were also treated with the indicated growth factors in DMEM containing [^{3}H]thymidine (1 μCi/ml, 1 μM) for 25 h to determine the percentage of labeled nuclei. Cells were fixed with 3.7% formalin and then processed for autoradiography as described previously [26]. In the quiescent cultures used in the present experiments the numbers of labeled nuclei were as low as 1%.

Measurement of glucose uptake. Quiescent cultures of 3T3 cells grown in 35-mm dishes were washed twice with DMEM and then incubated with various concentrations of growth factors in 1 ml of DMEM at 37°C. After the indicated incubation period, the cultures were washed twice with PBS, and then the medium was replenished with 1 ml of prewarmed glucose-free DMEM containing 2-deoxy[^{3}H]glucose (1 μCi/ml) or 3-*O*-methyl[^{3}H]glucose (1 μCi/ml) at the indicated concentrations, as described previously [27]. The cells were then incubated at 37°C for 5–15 min or 1–2 min, respectively. During that period, incorporation of the radioactivity into the cells was observed to be linear. Cells were then washed twice with cold PBS, and the radioactive materials inside the cells were extracted with 5% trichloroacetic acid.

Measurement of uridine uptake. Quiescent 3T3 cells were incubated with growth factors, as described above, in 1 ml of DMEM for 3 h and then the cells were further incubated in duplicate with 1 ml of fresh DMEM containing [^{3}H]uridine (0.5 μCi/ml, 1 μM) for 15 min at 37°C. The cells were washed twice with cold PBS, and 5% trichloroacetic acid-soluble radioactivity in the cells was extracted and counted.

RNA blot analysis. Total cellular RNA was isolated from guanidine isocyanate lysates of growth factor-treated and untreated resting 3T3 cells by the procedure of Maniatis et al. [28], denatured, run on a 1.5% agarose gel containing 2.2 M formaldehyde, and transfered to a nylon membrane (Hybond-N, Amersham). The membranes were hybridized with a ^{32}P-labeled glucose transporter cDNA in 50% formamide, 5 × SSPE, 5 × Denhardt's (0.05% bovine serum albumin/0.05% Ficoll/0.05% polyvinylpyrrolidone) and 100 μg/ml of denatured salmon testis DNA at 42°C for 20–40 h [28]. After hybridization, the membranes were washed with 2 × SSC containing 0.1% SDS at room temperature, and again with 0.2 × SSC containing 0.1% SDS at 45°C. The hybridized blots were exposed to an X-ray film at −70°C with an intensifying screen. The cDNA probe isolated from rat brain [22] was labeled with [α-^{32}P]dCTP by nick translation [28].

Protein was determined by the method of Lowry et al. with bovine serum albumin as a standard [29].

Results

Stimulation of DNA synthesis by serum or growth factors

DNA synthesis in G_0-arrested cells was reinitiated synchronously after a lag period for 12–15 h by the addition of fresh calf serum. This transition from the G_0

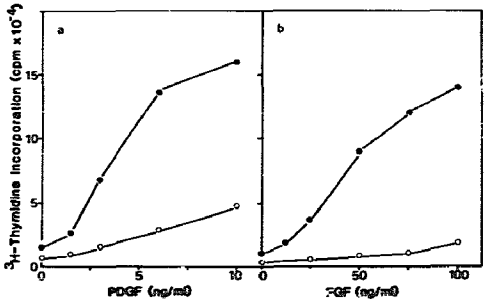

Fig. 1. Effects of PDGF and FGF on stimulation of DNA synthesis in 3T3 cells. (a) Quiescent cells were incubated for 24 h with 1 ml DMEM containing various concentrations of PDGF with (●) or without (○) 5% plasma, and the stimulation of DNA synthesis in the cells was measured. (b) Similar experiments performed with FGF. In this experiment, the radioactivity incorporated into the cells incubated with 10% FCS was $165 \cdot 10^3$ cpm/dish and the % labeled nuclei was 96%, while that of untreated culture was 1.5%.

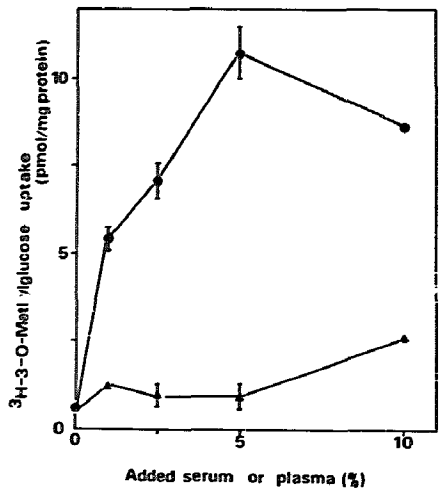

Fig. 2. Stimulation of 3-*O*-methylglucose uptake in quiescent cells. Quiescent 3T3 cells were incubated with 1 ml of DMEM containing various amounts of serum (●) or plasma (▲) at 37 °C for 3 h, and the uptake of 3-*O*-methyl[^{3}H]glucose (1 μCi/ml, 1 μM) at 37 °C for 2 min in the treated cells was determined as described in Materials and Methods.

phase to the S phase was stimulated by 5–10% calf serum, but platelet-poor plasma containing similar amounts of protein did not induce DNA synthesis in the cells. The complete stimulatory activity on DNA synthesis was recovered when PDGF, a major mitogenic peptide derived from blood platelets [2,3,30], was added together with 5% platelet-poor plasma to the quiescent cultures, although PDGF alone showed little activity at these concentrations (Fig. 1a). Similar results were obtained when FGF was used instead of PDGF (Fig. 1b). These results for Swiss 3T3 cells were consistent with the previous ones for Balb/C 3T3 cells [2], indicating that for serum-induced DNA synthesis in quiescent fibroblasts a combination of two independent growth factors, a competence factor and platelet-poor plasma containing progression factors, is required.

Stimulation of glucose transport by growth factors

To determine the effects of growth factors on the early change in the uptake of 3-*O*-methylglucose, cells were treated with various concentrations of either calf serum or platelet-poor plasma for 3 h at 37 °C. As shown in Fig. 2, serum-treatment greatly stimulated transport activity in the cells but platelet-poor plasma was far less effective suggesting an important role for platelet components in the regulation of glucose transport.

To examine this possibility, quiescent cells were treated with various concentrations of PDGF for 3 h, and changes in 2-deoxyglucose uptake were followed. Fig. 3a shows that uptake was stimulated by PDGF alone to the same level as that seen with 5% serum. Maximum stimulation was observed with 5–10 ng/ml of PDGF. FGF also stimulated hexose uptake in a dose dependent manner (Fig. 3b). Similar results were also obtained when 3-*O*-methylglucose was used in place of 2-deoxyglucose. The concentrations of PDGF and FGF required for induction of DNA synthesis were similar to those required for stimulation of glucose transport.

Increases in 2-deoxyglucose uptake in cells treated with serum (5%), PDGF (10 ng) or FGF (100 ng) were evident within 1 h after the addition of the growth factors, and achieved maximal levels after treatment for 3–5 h (Fig. 4). The transport activity stimulated by growth factors returned to a basal level after 20 h incubation (data not shown). The profiles for stimula-

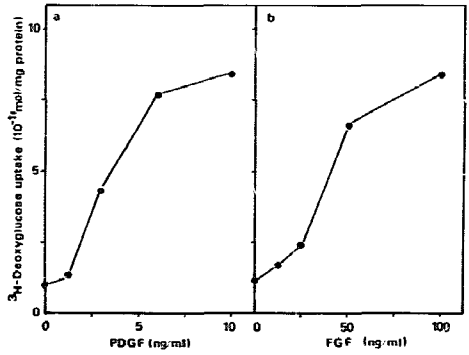

Fig. 3. Stimulation of 2-deoxyglucose uptake by PDGF or FGF in 3T3 cells. Quiescent 3T3 cells were incubated with various concentrations of PDGF (a) or FGF (b) in DMEM for 3 h at 37 °C, and 2-deoxyglucose uptake was measured. The radioactivity incorporated into cells, which had been treated with 5% serum for 3 h was $8.3 \cdot 10^{-11}$ mol/mg protein per 10 min.

tion of glucose transport in growth factor-treated cells were quite similar to those obtained with cultures stimulated with serum, DPGF and FGF, suggesting the existence of a common mechanism.

Effects of various growth factors on glucose and uridine uptake

As described above, PDGF or FGF alone stimulated glucose transport in quiescent 3T3 cells to the level seen with whole serum. In addition, a potent tumor promoter, PMA, also enhanced 2-deoxyglucose uptake activity in the cells under the same conditions (Table I). These three different growth promoting factors, PDGF, FGF and PMA, which stimulated 2-deoxyglucose uptake in G_0 3T3 cells, have been classified as competence growth factors [2,3]. By contrast the factors reported as progression factors, including platelet-poor plasma (5–10%), insulin (1 μg) and EGF (50–100 ng), exhibited much lower stimulatory activities.

To determine whether these characteristics of the growth factors are specific for the glucose transport system, we also studied their effects on uridine uptake. As expected [1,4,5,26], uridine uptake by cells activated with 5% serum for 3 h was increased about 4-fold (Table I). However, platelet-poor plasma stimulated uridine uptake similarly. Furthermore, all of the growth factors tested, including PDGF (10 ng), FGF (100 ng), PMA (50 ng), insulin (1 μg) and EGF (50–100 ng), enhanced uridine uptake about 3–4-fold. Thus in this case there were no significant differences in the activity of the two classes of growth factors.

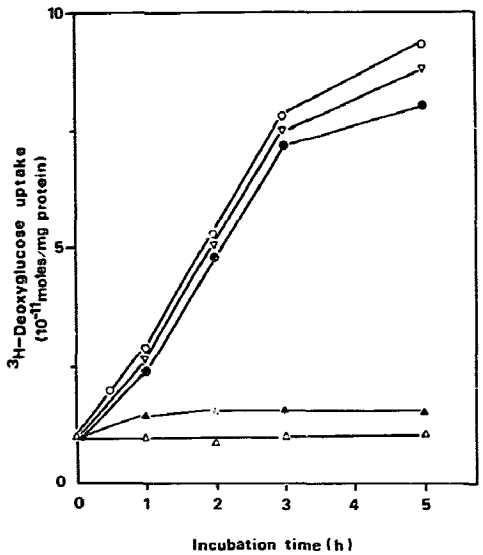

Fig. 4. Period of exposure to growth factors for stimulation of 2-deoxyglucose uptake. Quiescent 3T3 cells were incubated with 1 ml of DMEM containing 5% serum (○), 10 ng PDGF (●), 100 ng FGF (▽), 5% plasma (▲) or DMEM alone (△). After incubation for the indicated period, deoxy[^{3}H]glucose uptake for 10 min in the cells was determined.

TABLE I

Effects of growth factors on glucose and uridine uptake in 3T3 cells

Quiescent 3T3 cells were incubated with various growth factors in 1 ml DMEM for 3 h at 37°C, and the uptake of 2-deoxy[^{3}H]glucose (1 μCi/ml, 1 μM) for 10 min or [^{3}H]uridine (0.5 μCi/ml, 1 μM) for 15 min at 37°C was determined as described in Materials and Methods. The results are the means ± S.E. for 2–4 experiments. n.d., not determined.

Added growth factors		Deoxyglucose uptake (10^{-11} mol/mg protein)	Uridine uptake (10^{-10} mol/mg protein)
None		1.10 ± 0.08	0.62 ± 0.15
Serum	(5%)	7.68 ± 0.20	2.67 ± 0.6
Plasma	(5%)	1.45 ± 0.30	2.55 ± 0.35
PDGF	(10 ng)	7.50 ± 0.70	2.10 ± 0.5
FGF	(100 ng)	8.03 ± 0.50	2.00 ± 0.5
PMA	(10 ng)	6.13 ± 0.10	n.d.
	(50 ng)	6.33 ± 0.06	2.25 ± 0.6
Insulin	(1 μg)	2.17 ± 0.38	2.10 ± 0.2
EGF	(50 ng)	1.68 ± 0.40	1.55 ± 0.1
	(100 ng)	1.73 ± 0.27	1.50 ± 0.1

Characteristics of the stimulated glucose transport

The stimulated uptake of 2-deoxyglucose or 3-*O*-methylglucose was completely inhibited by either 0.5 μM cytochalasin B or 100 μM phroretin in cultures of both quiescent cells and cells stimulated with serum, PDGF, FGF or PMA (data not shown). However, phroridine (100 μM) a preferential inhibitor of the Na^+-dependent glucose transport system [19], showed only a small inhibitory effect (10–15%). These results, as well as the results of kinetic analysis described below, indicate that the glucose transport activity in both quiescent and stimulated 3T3 cells reflects a transport system involving a carrier-mediated facilitated diffusion, as described for other mammalian cells [17,20,31].

The results of the kinetic constants obtained from Lineweaver-Burk plots of 2-deoxyglucose uptake in different states of quiescence and on stimulation by various growth factors shows that the V_{max} for the uptake increased 5–6-fold in the culture stimulated by either serum, PDGF, FGF or PMA (data not shown). However, the K_m for the uptake in these cells was approximately 1–1.5 mM under all culture conditions. These V_{max} and K_m values for glucose uptake are within the range reported for other systems mediated by carrier-mediated facilitated diffusion [13,17].

To determine further the requirements for new protein and RNA synthesis in this stimulation by the growth factors, the effects of inhibitors of macromolecular synthesis were examined. In all cultures stimulated

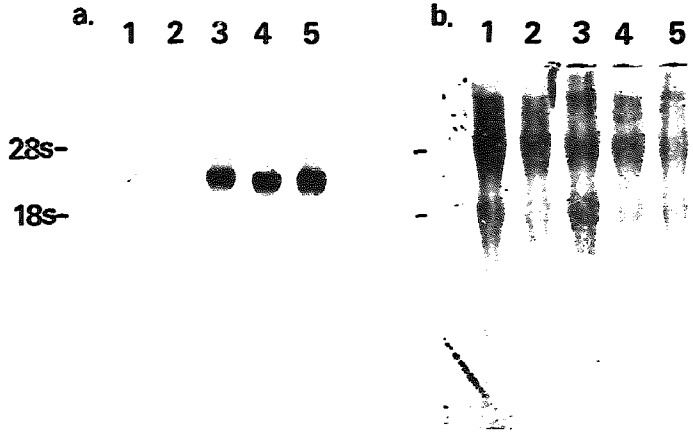

Fig. 5. RNA blot analysis of glucose transporter gene. Quiescent 3T3 cells were incubated with 5% serum in DMEM at 37 °C for 0, 0.5, 1, 2 and 5 h and the total RNA was extracted and separated on an agarose gel (20 μg/lane). The blotted filter was then hybridized with ^{32}P-labeled glucose transporter cDNA probe (a). After hybridization, the filter was stained with acridine orange (b).

with serum, PDGF, FGF or PMA, an early increase in glucose uptake was detectable and at 1 h of incubation was insensitive to both of the inhibitors (Table II). However, the uptake on stimulation for 3 h was inhibited about 70% by either cycloheximide or actinomycin D, suggesting that new protein and RNA synthesis are required for maximal glucose transport activity. It should also be noted that the residual activities after 3 h incubation with the growth factors in the presence of one of the inhibitors were nearly the same as those after 1 h incubation, that were insensitive to the inhibitors. These results demonstrate that the stimulation by growth factors of glucose transport activity involves macromolecular (protein and RNA) synthesis-independent and -dependent processes. DNA synthesis is not required for the stimulation, since the cells remain in the prereplicative phase.

Stimulation of expression of glucose transporter mRNA by growth factors

Changes in level of glucose transporter mRNA in quiescent 3T3 cells were also determined by RNA blot analysis. As shown in Fig. 5, an increase in glucose transporter mRNA (2.8 kb) occurred 1 h after serum stimulation, and reached a maximal level after 2–5 h of stimulation. In agreement with the results of glucose transport, PDGF, FGF and PMA also stimulated the accumulation of glucose transporter mRNA during 1–3

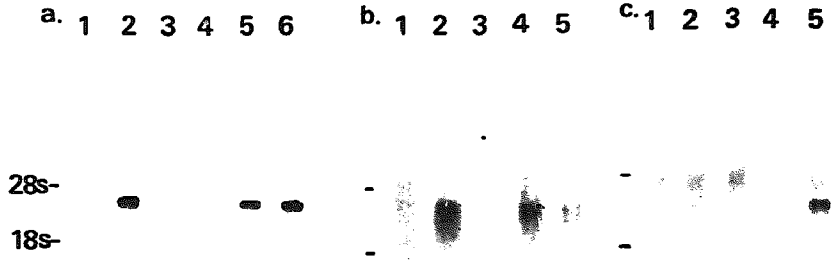

Fig. 6. Changes in glucose transporter mRNA levels in growth factors-treated 3T3 cells. Quiescent 3T3 cells were incubated for 3 h (a, b) or 20 h (c) with various growth factors as follows and changes in glucose transporter mRNA level were determined: (a): (1) DMEM, (2) 5% serum, (3) 5% plasma, (4) 1 μg insulin, (5) 100 ng FGF, (6) 25 ng PMA; (b): (1) DMEM, (2) 5% serum, (3) 5% plasma, (4) 10 ng PDGF, (5) 100 ng EGF; (c): (1) DMEM, (2) 5% serum, (3) 100 ng FGF, (4) 25 ng PMA, (5) 25 ng PMA, 3 h.

TABLE II

Effects of cycloheximide and actinomycin D on the increased uptake in 3T3 cells

Quiescent cells were incubated with DMEM containing the indicated growth factors and either cycloheximide (10 μg/ml) or actinomycin D (1 μg/ml). After incubation for 1 or 3 h at 37°C, deoxy[^{3}H]glucose uptake was determined. n.d., not determined.

Growth factors		Time (h)	Deoxy[^{3}H]glucose uptake (10^{-11} mol/mg protein)		
			−inhibitor	+CH	+Act. D
None		1.0	1.0	n.d.	n.d.
Serum	(5%)	1.0	2.6	2.6	2.2
PDGF	(10 ng)	1.0	3.0	3.1	3.6
FGF	(50 ng)	1.0	2.8	2.9	2.5
PMA	(25 ng)	1.0	2.7	2.3	2.1
None		3.0	1.1	n.d.	n.d.
Serum	(5%)	3.0	7.4	3.2	2.4
PDGF	(10 ng)	3.0	7.3	3.1	2.8
FGF	(50 ng)	3.0	7.3	2.5	2.4
PMA	(25 ng)	3.0	5.2	2.4	2.2

h incubation, but they became undetectable by 20 h (Fig. 6). In contrast, plasma, insulin and EGF failed to enhance the mRNA level.

The increased level of this mRNA in serum-treated 3T3 cells was completely inhibited by actinomycin D, suggesting that this increase is due to, at least in part, enhanced transcription of the gene (Fig. 7). However, mRNA accumulations induced by serum, FGF or PMA were not significantly affected by a protein synthesis inhibitor, cycloheximide.

TABLE III

Effect of pretreatment with PMA on the growth factor-dependent increase in deoxy[^{3}H]glucose uptake in 3T3 cells

Quiescent 3T3 cells were preincubated with 25 ng/ml PMA in DMEM for 24 h at 37°C. The cells were washed and then further incubated for 3 h with the indicated growth factors in DMEM. Deoxy[^{3}H]glucose uptake in the culture was determined as described in Materials and Methods.

Growth factors	Deoxy[^{3}H]glucose uptake (10^{-11} mol/mg protein)	
	untreated cells	PMA-treated cells
None	0.60	0.65
PMA (25 ng)	4.07	0.73
OAG (25 μg)	3.90	0.90
Serum (5%)	6.33	4.53
FGF (50 ng)	6.50	5.20
PDGF (10 ng)	6.27	5.60

Studies with PMA-pretreated cells

We have shown that serum, PDGF and FGF as well as PMA stimulate glucose transport activity and expression of glucose transporter mRNA with similar characteristics in quiescent 3T3 cells. It has already been reported that PDGF and FGF elicit the rapid formation of diacylglycerol, activation of protein kinase C and Ca^{2+} mobilization when added to quiescent of cells, including Swiss 3T3 cells [32–34]. Consequently, it was feasible that activation of protein kinase C initiated by the PDGF- or FGF-induced breakdown of phosphatidylinositides led to stimulation of glucose transport activity through direct activation of protein kinase C.

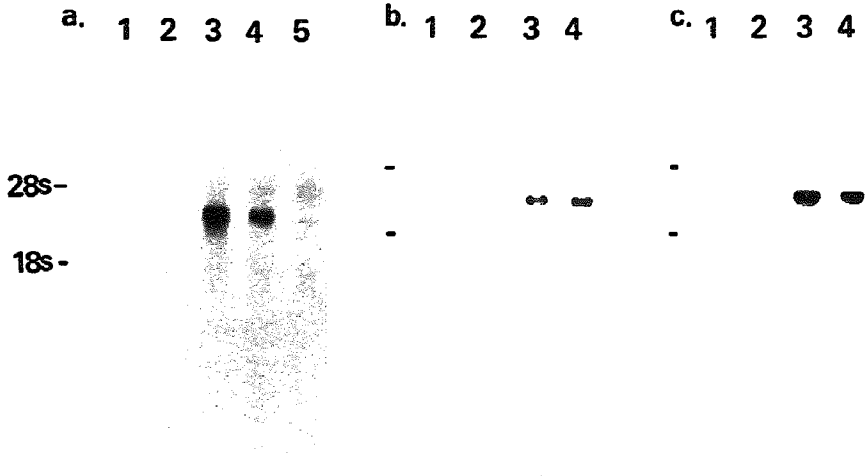

Fig. 7. Effects of metabolic inhibitors on expression of glucose transporter mRNA. Resting 3T3 cells were treated with 5% serum (a), 25 ng PMA (b) or 100 ng FGF (c) for 3 h in the presence or absence of the indicated inhibitors and expression of glucose transporter mRNA was determined. Lane 1, DMEM, lane 2, DMEM + CH (20 μg/ml), lane 3, growth factor, lane 4, growth factor + CH, lane 5 in a, serum + actinomycin D (1 μg/ml).

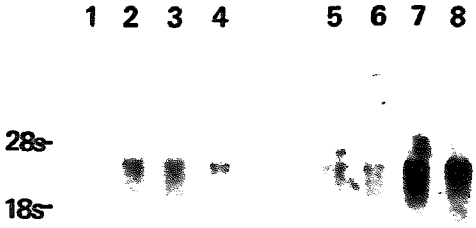

Fig. 8. Effects of PMA-pretreatment on expression of glucose transporter mRNA. Untreated (lane 1–4) or PMA-treated (lane 5–8) 3T3 cells as described in Table II were further treated with DMEM alone (1, 5), 25 ng PMA (2, 6), 5% serum (3, 7), or 100 ng FGF (4, 8) for 3 h and levels of glucose transporter mRNA of these cells were determined.

This mechanism has been implicated in the cases of the phosphorylation of several cellular proteins [35–37].

Blackshear et al. [37] and Kaibuchi et al. [34] recently reported that protein kinase C activity in 3T3 cells can be almost completely abolished by prolonged exposure to phorbol esters. When quiescent 3T3 cells were exposed to various concentrations of PMA for 24 h in DMEM, PMA-dependent stimulation of glucose uptake was completely abolished (Table III), as described recently by Kitagawa et al. [38]. This decrease in the PMA response was observed in cells pretreated with as little as 10 ng/ml of PMA. In the protein kinase C-deficient cultures, OAG-dependent stimulation of glucose uptake was also apparently suppressed. However, glucose uptake by these cells was still enhanced by serum, PDGF and FGF. Similar results were obtained when the cells were pretreated with PDBu, an analogue of PMA (data not shown).

In 3T3 cells pretreated with PMA, the PMA-inducible increase in glucose transporter mRNA also became undetectable (Fig. 8). However, the mRNA level was still increased by serum or FGF, demonstrating the presence of a PMA-independent pathway to regulate glucose transport as well as the accumulation of transporter mRNA.

Discussion

In the present study there are three principal findings. First, an early increase in glucose transport in quiescent 3T3 cells is caused by PDGF or FGF alone, and does not require the simultaneous presence of plasma components. This is in apparent contrast to the effect on DNA synthesis in the cells, for which the presence of platelet-poor plasma with either PDGF or FGF is required (Fig. 1). PDGF and FGF stimulated glucose transport activity in 3T3 cells to the same extent as serum (Fig. 3). The characteristics of the increased transport were also quite similar to those obtained with serum: the time courses for the stimulation (Fig. 4), changes in kinetic constants with increased V_{max} values without changes in apparent K_m, and sensitivity of the increased activity to inhibitors of macromolecular synthesis (Table II). In addition, the concentrations of PDGF and FGF required for stimulation of glucose transport and DNA synthesis are well correlated (Figs. 1 and 3). These results are not conclusive but strongly suggest that the early increase in glucose transport coupled to the transition from the quiescent state (G_0) to the proliferative state is mainly regulated by a single peptide growth factor such as PDGF and FGF.

The stimulation of glucose transport is mediated by growth factors which have been reported to have competence activities [2,3], including serum, PDGF and FGF as well as PMA, but growth factors classified as progression factors, i.e., plasma, insulin and EGF, have much less effect under the same experimental conditions (Table I). In contrast to these results, uridine uptake, another early permeability change in the same quiescent cultures [5], is stimulated similarly by competence growth factors and progression factors. Of particular relevance to the present results is the observation of Owen et al., that amino acid transport system A in Balb 3T3 cells is stimulated by PDGF alone but not by plasma components [39].

Second, we observed that the stimulated glucose transport activity in the quiescent 3T3 cells was accompanied by an enhanced level of glucose transporter mRNA evident after 1 h of stimulation (Figs. 5 and 6). This could be due to an increase in transcription of the gene, since the specific inhibitor, actinomycin D, inhibited the increase in the transporter mRNA as well as maximal stimulation of the glucose transport (Fig. 7, Table II). Furthermore, this enhancement of the transporter mRNA level was not greatly affected by cycloheximide, suggesting that a newly synthesized cellular protein is not required for the accumulation of this mRNA. These facts together with the results from kinetic analysis of the transport suggest that the cell-cycle dependent stimulation of glucose transport by growth factors such as PDGF and FGF are partly regulated at the transcriptional level of the transporter, although more direct analysis of the transcription is required.

Third, we have shown that there are at least two independent pathways for growth factor-induced stimulation of glucose transport in quiescent 3T3 cells. One of these pathways probably involves activation of protein kinase C. As described previously [13,14], phorbol

esters such as PMA (Table I) and PDBu (data not shown) stimulated glucose transport, and the characteristics of the stimulated activity were found to be similar to those induced by serum, PDGF and FGF. OAG, a synthetic analogue of diacylglycerol, enhanced the transport activity like PMA (Table III). However, glucose transort can no longer be stimulated by PMA or OAG in cells that are protein kinase C deficient [34,37].

As described, PDGF and FGF generate diacylglycerol as well as inositol phosphates by stimulating the hydrolysis of phosphoinositides [32–37]. However, serum, PDGF and FGF are able to stimulate glucose transport in PMA-pretreated, protein kinase C-deficient 3T3 cells, as reported recently [38]. These two different pathways also seem to be operating at the level of gene expression (Fig. 8). These results indicate the presence of a protein kinase C-independent pathway for control of glucose transporter mRNA level and transport activity; it also implies that this pathway mainly operates for the stimulation of glucose transport during the course of serum-induced mitogenesis.

The findings reported here have important implications for future studies on the regulatory mechanisms of glucose transport and cell proliferation. As reported previously in serum or PMA-induced fibroblasts [5–7,14], a PDGF or FGF-induced increase in glucose transport in 3T3 cells consist of an early macromolecular synthesis-insensitive process and a later sensitive step (Table II). In fat cells, insulin rapidly stimulates glucose transport, mainly due to translocation of the glucose transporter from cellular pools to the plasma membrane [8,9]. These results taken together may indicate that the stimulation of glucose transport by growth factors could be controlled by increasing the number of active glucose transporters in the plasma membrane due to early (within 1 h) translocation and the later (1–3 h) biosynthesis of the transporter, though they have not been measured directly. Furthermore, of interest is the fact that the regulation of proto-oncogenes (*c-fos*, *c-myc*), since their expressions are also stimulated by competence growth factors in fibroblasts [39–42], was also confirmed under our experimental conditions (Kitagawa, T., Tanaka, M. and Akamatsu, Y., unpublished data). Thus, it is important to determine further cellular components which regulate glucose transporter mRNA level.

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After submission of the manuscript, a rapid activation of the glucose transporter gene by growth factors including PDGF, FGF, EGF and PMA at transcriptional level was observed by Hiraki, Y. et al. (J. Biol. Chem. 263, 13655–13662 (1988). Similar results were also reported by Rollins, B.J. et al. (J. Biol. Chem. 263, 16523–16526 (1988).

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