Transient Effect of Platelet-derived Growth Factor on GLUT4 Translocation in 3T3-L1 Adipocytes*  

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We earlier developed a novel method to detect translocation of the glucose transporter (GLUT) directly and simply using c-MYC epitope-tagged GLUT (GLUT4MYC). To define the effect of platelet-derived growth factor (PDGF) on glucose transport in 3T3-L1 adipocytes, we investigated the PDGF- and insulin-induced glucose uptake, translocation of glucose transporters, and phosphatidylinositol (PI) 3-kinase activity in 3T3-L1, 3T3-L1GLUT4MYC, and 3T3-L1GLUT1MYC adipocytes. Insulin and PDGF stimulated glucose uptake by 9–10- and 5.5–6.5-fold, respectively, in both 3T3-L1 and 3T3-L1GLUT4MYC adipocytes. Exogenous GLUT4MYC expression led to enhanced PDGF-induced glucose transport. In 3T3-L1GLUT4MYC adipocytes, insulin and PDGF induced an 8- and 5-fold increase in GLUT4MYC translocation, respectively, determined in a cell-surface anti-c-MYC antibody binding assay. This PDGF-induced GLUT4MYC translocation was further demonstrated with fluorescence detection. In contrast, PDGF stimulated a 2-fold increase of GLUT1MYC translocation and 2.5-fold increase of glucose uptake in 3T3-L1GLUT1MYC adipocytes. The PDGF-induced GLUT4MYC translocation, glucose uptake, and PI 3-kinase activity were maximal (100%) at 5–10 min and thereafter rapidly declined to 40, 30, and 12%, respectively, within 60 min, a time when effects of insulin were maximal. Wortmannin (0.1 μM) abolished PDGF-induced GLUT4MYC translocation and glucose uptake in 3T3-L1GLUT4MYC adipocytes. These results suggest that PDGF can transiently trigger the translocation of GLUT4 and stimulate glucose uptake by translocation of both GLUT4 and GLUT1 in a PI 3-kinase-dependent signaling pathway in 3T3-L1 adipocytes.

The insulin signaling pathway mediating the glucose transport is not fully understood. Translocation of GLUT4 from an intracellular pool to the plasma membrane is thought to be a major mechanism of glucose uptake in response to insulin in insulin-sensitive tissues (1–3). We (4) and others (5–8) found that PI 3-kinase activation is essential for insulin-stimulated glucose uptake. Platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) activate many of the same signaling cascades as does insulin. One of the most prominent shared pathways is phosphatidylinositol 3-kinase. Therefore, the question arose as to whether PDGF or EGF would trigger GLUT4 translocation by the activation of PI 3-kinase. We developed a sensitive immunological method that can detect c-MYC epitope-tagged GLUT4 (GLUT4MYC) on the cell surface, directly and quantitatively (9). By using this method, we have found that PDGF and EGF did trigger the GLUT4 translocation to the plasma membrane in CHO and 3T3-L1 adipocytes by a signaling pathway involving phosphatidylinositol 3-kinase (PI 3-kinase, p85/p110 heterodimer type) (10, 11). We considered that PDGF and EGF as well as insulin may have latent potential to trigger GLUT4 translocation by activation of PI 3-kinase in cultured cells. PDGF- or EGF-triggered GLUT4 translocation has been reported by other research groups (12, 13). However, there is a great deal of debate on the effect of PDGF on glucose transport in 3T3-L1 adipocytes. It was reported that PDGF has no significant effect or only a minimal effect on glucose transport in 3T3-L1 adipocytes (14, 15), and other workers (16–18) suggested that PDGF can stimulate glucose uptake by the translocation of GLUT1 instead of GLUT4, as based on findings that PDGF had no detectable effect on GLUT4 translocation in 3T3-L1 adipocytes, determined using fractionation procedures or photoaffinity labeling. They found that PDGF did not stimulate GLUT4 translocation even though PDGF did stimulate PI 3-kinase to an extent similar to that seen with insulin in 3T3-L1 adipocytes; they proposed that activation of PI 3-kinase alone is not sufficient to stimulate GLUT4 translocation to the plasma membrane (14, 18).

To understand better the pathophysiology of insulin-resistant states, it is crucial to identify key molecular components necessary to mediate insulin signaling. Since insulin and PDGF are growth factors, there are overlaps between signal transduction pathways. Understanding the effect of PDGF on glucose transport may aid in identifying insulin signaling pathway mediating the glucose transport. Murine 3T3-L1 cells express both insulin and PDGF receptors and represent an in vitro model for insulin-induced glucose transport (19). To extend our previous study on the effect of PDGF on glucose transport and the role of PI 3-kinase in the PDGF-induced glucose transport, we compared the effects of insulin and PDGF on the stimulation of glucose transport, translocations of glucose transporters and PI 3-kinase activity in 3T3-L1, 3T3-L1GLUT4MYC, and 3T3-L1GLUT1MYC adipocytes. We found that 1) PDGF stimulated glucose uptake in both 3T3-L1 fibro-
on glucose uptake. Cells were stimulated with 100 nM insulin or 50 ng/ml PDGF-BB for 10 min at 37 °C. The 2-deoxyglucose uptake was measured as described (22, 23). Cells were stimulated with 100 nM insulin or 50 ng/ml PDGF-BB for 10 min at 37 °C. The 2-deoxyglucose uptake was measured as described under "Experimental Procedures." Values represent means ± S.E. of three separate experiments done in triplicate.

EXPERIMENTAL PROCEDURES

Materials—Insulin was purchased from Sigma and PDGF-BB was purchased from PeproTech Ltd. (London, UK). Dulbecco's modified Eagle's medium was obtained from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). Calf bovine serum was obtained from ICN Biomedicals, Inc. (Costa Mesa, CA), and fetal bovine serum was obtained from Life Technologies, Inc. A hybridoma of monoclonal antibody (9E10) against human c-MYC was obtained from the American Type Culture Collection. Antibodies to C terminus of GLUT4 were prepared by immunizing rabbit with C-terminal 30 amino acids of rat GLUT4. Antibodies to C terminus of GLUT1 were purchased from Chemicon International Inc. (Temecula, CA). Secondary horseshadish peroxidase-conjugated goat anti-mouse IgG and anti-rabbit IgG were obtained from BioSource (Costa Mesa, CA). An anti-phosphotyrosine antibody (PY20) was purchased from Transduction Laboratories (Lexington, KY). Tyramide signal amplification kit (green) for immunohistochemistry was purchased from Moravek Biochemicals. Inc. (Brea, CA). All other biochemicals were obtained from Sigma.

Cell Culture—3T3-L1 murine fibroblasts were obtained from the American Type Culture Collection (Rockville, MD). The cell lines used in this study were 3T3-L1 fibroblasts, 3T3-L1GLUT4MYC, a 3T3-L1 fibroblast line stably overexpressing GLUT4MYC, constructed by inserting a human c-MYC epitope (14 amino acids) into the first ectodomain of GLUT4, and 3T3-L1GLUT1MYC, a 3T3-L1 fibroblast line stably overexpressing GLUT1MYC (9, 20). The 3T3-L1, 3T3-L1GLUT4MYC, and 3T3-L1GLUT1MYC fibroblasts were cultured in Dulbecco's modified Eagle's medium, 10% calf bovine serum and induced to differentiate into adipocytes, as described (21). The adipocytes were used between 7 and 9 days after the initiation of differentiation, a time when more than 90% of the cells had an adipocyte phenotype.

Western Blot Analyses of GLUTMYC Protein—Subcellular fractions from 3T3-L1, 3T3-L1GLUT4MYC, and 3T3-L1GLUT1MYC adipocytes were prepared as described (20). Equal amounts of protein from the same fraction number of different cells were resolved by 10% SDS-polyacrylamide gel electrophoresis and transferred by electroblootting onto nitrocellulose membrane (Schleicher & Schuell). Blots were probed with mouse monoclonal antibodies to the human c-MYC epitope of 14 amino acids (9E10), rabbit polyclonal antibodies to C terminus of GLUT4, or rabbit polyclonal antibodies to C terminus of GLUT1. Proteins were visualized using enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech).

Cell Surface Anti-c-MYC Antibody Binding Assay (GLUTMYC Translocation Assay)—The 3T3-L1GLUT4MYC or 3T3-L1GLUT1MYC cells in 24-well plates were incubated in 500 μl of Krebs-Ringer/Hepes (KRH) buffer (136 mM NaCl, 4.7 mM KCl, 1.25 mM CaCl2, 1.25 mM MgSO4, 20 mM Hepes, pH 7.4, and 2 mg/ml bovine serum albumin) for 30 min at 37 °C and then with indicated concentrations of ligands for indicated periods at 37 °C. GLUTMYC translocation was measured, as described (9).

2-Deoxyglucose Uptake Measurement—Cells cultured in Dulbecco's modified Eagle's medium, 10% fetal calf serum medium with 24-well plates were washed once with KRH buffer and incubated for 30 min in KRH buffer and then treated with indicated concentrations of ligands for 10 min at 37 °C. 2-Deoxy[3H]glucose uptake was measured by incubating cells with the deoxy[3H]glucose for 10 min, as described (22, 23).

Sudan III Stain—Cells in a 6-well plate were incubated with 5 ml of 50% ethanol for 2 min at room temperature and then stained with 1 ml of 2% Sudan III in 70% ethanol for 30 min at 37 °C. After washing the cells once with 5 ml of 50% ethanol and twice with 5 ml of H2O at room temperature, photographs were taken.

Immunostaining and Fluorescence Microscopy—GLUTMYC protein staining was done using the tyramide signal amplification-direct procedure following the manufacturer's instructions (NEN Life Science Products). Briefly, 3T3-L1, 3T3-L1GLUT4MYC, and 3T3-L1GLUT1MYC cells were grown in a 6-well plate and converted to adipocytes. The adipocytes were incubated in 1 ml of KRH buffer for 30 min at 37 °C, and then indicated concentrations of ligands were added for 10 min at 37 °C. After fixation with 2% paraformaldehyde/phosphate-buffered saline (137 mM NaCl, 8.1 mM Na2HPO4, 2.7 mM KCl, 1.5 mM KH2PO4, pH 7.4) at room temperature for 20 min, cells were washed three times with phosphate-buffered saline, treated with 100 μl of glycine/phosphate-buffered saline at room temperature for 15 min, and incubated with TMB blocking buffer.
(0.1 m Tris-HCl, pH 7.5, 0.15 m NaCl, 0.5% Blocking Reagent supplied in a kit) at room temperature for 30 min. They were then incubated with the anti-c-MYC antibodies (1:20 dilution with TNB blocking buffer) at 37 °C for 30 min and then further incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (1:20 dilution with TNB blocking buffer) at 37 °C for 30 min. Each antibody incubation was followed by three washes for 5 min each with TNT buffer (0.1 m Tris-HCl, pH 7.5, 0.15 m NaCl, 0.05% Tween 20), at room temperature. Cells then were incubated with fluorophore tyramide (1:50 dilution with amplification buffer) for 7 min at room temperature in a dark, moist chamber, washed three times for 10 min each in TNT buffer at room temperature and visualized under a fluorescence microscope (Olympus BX50).

**PI 3-Kinase Assay**—Preparation of cell lysates and PI 3-kinase assay were done as described (24). Briefly, total cell lysates were prepared with buffer containing 1% Nonidet P-40 after treatment with the indicated concentrations of insulin or PDGF and precipitated with the PY20 antibodies and protein A-Sepharose CL-4B (Amersham Pharmacia Biotech). The immunoprecipitates were subjected to PI 3-kinase assay. The radioactive spots on TLC plates were quantified using a Bio-image analyzer BAS1500 (Fuji Film Institution).

**RESULTS**

**Effects of PDGF and Insulin on Glucose Uptake in 3T3-L1 Fibroblasts and Adipocytes**—To determine whether PDGF has any effect on glucose uptake, PDGF-induced glucose uptake was investigated using both 3T3-L1 fibroblasts and 3T3-L1 adipocytes. The *upper panel* of Fig. 1A shows the result of Sudan III staining. Few lipid droplets were seen in fibroblasts, and many lipid droplets appeared after induction, suggesting the cells were well differentiated into adipocytes. The *lower panel* of Fig. 1A shows the effects of PDGF and insulin on glucose uptake. In 3T3-L1 fibroblasts, a similar stimulation of glucose uptake was induced by PDGF (about 2-fold) and insulin (about 2-fold), an effect due to the translocation of GLUT1 from an intracellular pool to the plasma membrane (25, 26). In 3T3-L1 adipocytes, however, PDGF exerted a 6-fold stimulation of glucose uptake, even though this stimulation reached only 60% of the maximal effect of insulin (10-fold). This suggested that another glucose transporter, probably GLUT4, may be involved in the PDGF-induced glucose uptake, in addition to GLUT1, since the translocation of GLUT1 only caused about 2-fold stimulation of glucose uptake (27). To eliminate the possibility that the 3T3-L1 cells from different origins give different responses to PDGF, we also determined the PDGF-induced glucose uptake in another 3T3-L1 cell line (kindly provided by Dr. Morris Birnbaum, University of Pennsylvania). This 3T3-L1 cell line (named 3T3-L1-B) responded similarly to our 3T3-L1 cells (Fig. 1, A and B).

To confirm whether GLUT4 was responsible for the PDGF-induced glucose uptake, 3T3-L1 adipocytes stably overexpressing either GLUT4MYC or GLUT1MYC were evaluated.

**GLUTMYC Translocation and Glucose Uptake by PDGF and Insulin**—To examine which glucose transporter is responsible for the PDGF-induced glucose uptake, 3T3-L1 cell lines stably overexpressing either GLUT4MYC or GLUT1MYC were established and cell-surface anti-c-MYC antibody binding assay was done. As shown in Fig. 2A, the overexpression of GLUT4MYC or GLUT1MYC in 3T3-L1 cells was confirmed by immunoblotting with anti-c-MYC antibodies. In 3T3-L1GLUT4MYC adipocytes, the total expression of GLUT4 (endogenous GLUT4 plus GLUT4MYC) was approximately 2.5-fold over control (3T3-L1 adipocytes) as detected with antibodies C-terminal to GLUT4. In 3T3-L1GLUT1MYC adipocytes, the total GLUT1 (endogenous GLUT1 plus GLUT1MYC) was increased by 7-10-fold compared with endogenous GLUT1 as determined with antibodies C-terminal to GLUT1. After transfection with GLUT4MYC or GLUT1MYC, the adipocytes showed an enhanced increase in insulin- or PDGF-induced glucose transport compared with parent 3T3-L1 cells (Fig. 2B). As shown in Fig. 2C, PDGF led to a 5-fold increase of GLUT4MYC translocation in 3T3-L1 GLUT4MYC adipocytes and a 2-fold increase of GLUT1MYC translocation in 3T3-L1 GLUT1MYC adipocytes, whereas the parent 3T3-L1 adipocytes showed no response to either PDGF or insulin in the anti-c-MYC binding assay. These results suggest that PDGF can trigger the translocation of both GLUT4 and GLUT1 to the plasma membrane and exert the stimulation of glucose uptake in 3T3-L1 adipocytes. Also, the PDGF-stimulated GLUT4MYC translocation...
and glucose uptake were in a dose-dependent manner in 3T3-L1 GLUT4MYC adipocytes. However, PDGF only induced about 60% maximal effect of insulin on glucose transport with 10 min stimulation (Fig. 3).

Immunostaining and fluorescence microscopy were also used to determine further the effect of PDGF on translocation of glucose transporters in 3T3-L1GLUT4MYC and 3T3-L1GLUT1MYC adipocytes. As shown in Fig. 4, an increase in plasma membrane-associated fluorescence staining, as visualized as a ring around the cell, was induced by PDGF and insulin in both 3T3-L1GLUT4MYC and 3T3-L1GLUT1MYC adipocytes, whereas the controls showed only nonspecific staining without an enhanced staining in plasma membrane, indicating that PDGF stimulated translocations of GLUT4MYC and GLUT1MYC to the plasma membrane in 3T3-L1 adipocytes. As shown in Fig. 4, F and I, insulin triggered GLUT4MYC translocation in almost all the adipocytes. However, approximately 60% of cells showed positive staining with PDGF treatment, compared with findings in the case of insulin stimulation (Fig. 4, D–I) (see “Discussion”). These results were consistent with findings that PDGF induced approximately 60% of maximal effects of insulin on glucose uptake and translocation (Figs. 2 and 3).

Taken together, these results suggested that PDGF does exert stimulation of the 2-deoxyglucose uptake, and the PDGF-induced glucose uptake was shared by GLUT4 and GLUT1 transporters in 3T3-L1 adipocytes.

**Transient Effect of PDGF on GLUT4MYC Translocation and PI 3-Kinase Activation in 3T3-L1 Adipocytes**—It was reported that PDGF transiently activated PI 3-kinase in 3T3-L1 adipocytes (28). We reported that PDGF triggered GLUT4 translocation by a PI 3-kinase-dependent signaling pathway in CHO cells (11). To investigate the relationship between glucose transport and PI 3-kinase activity stimulated by insulin and PDGF in 3T3-L1 adipocytes, the time courses for insulin- and PDGF-induced glucose uptake, translocations of transporters, and PI 3-kinase activity were studied using 3T3-L1GLUT4MYC and 3T3-L1GLUT1MYC adipocytes. In 3T3-L1GLUT4MYC adipocytes, as shown in Fig. 5, A, B and E, PDGF-induced glucose uptake, GLUT4MYC translocation, and PI 3-kinase activity were maximal (100% of maximal effects of PDGF) at 5–10 min and thereafter rapidly declined to 30, 40, and 12%, respectively, within 60 min, a time when maximal effects of insulin on glucose uptake, GLUT4MYC translocation, and PI 3-kinase activity were observed. On the other hand, even though the maximal activity of PI 3-kinase induced by PDGF was about 2.1-fold higher than that stimulated by insulin (Fig. 5E), the PDGF stimulated less glucose uptake and GLUT4MYC translocation compared with that stimulated by insulin in 3T3-L1GLUT4MYC adipocytes (Figs. 2 and 3). This result suggested that the different ability of insulin and PDGF to activate PI 3-kinase and PI 3-kinase activation only is not sufficient to mediate the full effect of insulin on glucose transport (see “Discussion”).

Some differences in GLUT1MYC translocation and glucose uptake were observed in case of 3T3-L1GLUT1MYC adipocytes. As shown in Fig. 5, C and D, PDGF-induced GLUT1MYC translocation and glucose uptake reached maximal levels (100%) at 5–10 min and then declined slowly to 85 and 65%, respectively, within 60 min. In the insulin-stimulated state, the maximal levels of GLUT1MYC translocation and glucose uptake were observed to occur within 60 min, similar to findings in case of 3T3-L1GLUT4MYC adipocytes. Therefore, there are some differences between insulin-induced and PDGF-induced translocations of glucose transporters and different regulations in recycling of GLUT4 and GLUT1 glucose transporters.

PDGF stimulated the parallel translocations of glucose transporters and activation of PI 3-kinase in time courses, thereby suggesting the involvement of PI 3-kinase in PDGF-induced glucose transport. To confirm this, wortmannin was used in the experiments.

**Effects of Wortmannin on PDGF-induced GLUT4MYC**
Translocation and Glucose Uptake—Wortmannin is a specific and cell-permeable inhibitor of PI 3-kinase activity and is used widely in PI 3-kinase-related research (29). To determine if PI 3-kinase is involved in PDGF-induced GLUT4 translocation, wortmannin treatment was given prior to PDGF stimulation. As shown in Fig. 6, pretreatment with 0.1 mM wortmannin abolished PDGF-induced GLUT4MYC translocation and glucose uptake in 3T3-L1GLUT4MYC adipocytes. Therefore, effects of PDGF on GLUT4 translocation and glucose uptake depend on PI 3-kinase activity in 3T3-L1 adipocytes.

DISCUSSION

Insulin stimulates GLUT4 translocation and glucose uptake by a PI 3-kinase-dependent signaling pathway. We and others (10–13) have demonstrated that PDGF and EGF can also stimulate GLUT4 translocation and glucose uptake by a wortmannin-sensitive pathway in CHO cells and adipocytes. To investigate further the effect of PDGF on glucose transport, we examined glucose uptake and translocation of glucose transporters stimulated by PDGF in 3T3-L1 adipocytes that stably overexpressed either GLUT4MYC or GLUT1MYC.

Our observations strongly support the conclusion that PDGF can stimulate the translocation of GLUT4 and glucose uptake in 3T3-L1 adipocytes. First, when 3T3-L1 adipocytes were stimulated with PDGF, a 6.5-fold stimulation of the 2-deoxyglucose uptake was observed, whereas the translocation of GLUT1 caused only a 2–3-fold increase of glucose uptake (27). In addition, we also found that only a 2.5-fold increase of the 2-deoxyglucose uptake was obtained by PDGF treatment in 3T3-L1GLUT1MYC adipocytes (Fig. 2B). To eliminate the possibility that 3T3-L1 cells from different origins give different responses to PDGF and PDGF obtained from different origins causes different reactions, the 3T3-L1 cells and PDGF-BB from other origins also were used in experiments. Similar results of glucose uptake induced by PDGF were obtained using our cells (3T3-L1) and those of other investigators (3T3-L1-B) (Fig. 1, A and B). A 5-fold stimulation of the glucose uptake was also induced by PDGF-BB obtained from another provider (Austral Biological) in our 3T3-L1 adipocytes (data not shown). These results confirmed that there was no problem in the 3T3-L1 cell line and the PDGF-BB product used in present study. Thus, it is reasonable to assume that GLUT4 is involved in PDGF-induced glucose uptake. Second, exogenous GLUT4MYC expression led to enhanced PDGF-induced glucose transport. About double the amount of glucose uptake was stimulated by PDGF in 3T3-L1GLUT4MYC adipocytes, compared with that observed in parent 3T3-L1 adipocytes (Fig. 2B). However, overexpressing GLUT4MYC did not increase the fold stimulation induced by PDGF or insulin. In 3T3-L1GLUT4MYC adipocytes, PDGF stimulated a 5.7-fold increase in glucose uptake similar to that observed in parent 3T3-L1 adipocytes (Fig. 2B). Furthermore, cell-surface anti-c-MYC antibody binding assay showed that PDGF induced a 5-fold increase of GLUT4MYC translocation in 3T3-L1GLUT4MYC adipocytes and a 2-fold increase of GLUT1MYC translocation in 3T3-
L1GLUT1MYC adipocytes (Fig. 2C). Finally, immunostaining and fluorescence microscopy also showed an increase in plasma membrane-associated fluorescence staining after treatment with PDGF in 3T3-L1GLUT4MYC adipocytes (Fig. 4). These results strongly indicate that PDGF can stimulate the translocation of GLUT4 transporter and glucose uptake in 3T3-L1 adipocytes.

The present study suggested that the effect of PDGF on glucose uptake is transient and reaches the peak between 5 and 10 min after exposure to PDGF in 3T3-L1 adipocytes. It was reported that PDGF had only a limited effect on glucose uptake based on the experiments done with 15 min exposure to PDGF after 3 h incubation of KRBH/BSA buffer (14) or 20 min exposure to PDGF after overnight starvation of serum-free medium (18). It was possible to obtain more glucose uptake induced by PDGF in their cells if a shorter exposure to PDGF was performed. Furthermore, we only incubated the adipocytes with KRH buffer for 30 min before PDGF stimulation. The differences in the condition of the cell and the experimental procedure may contribute to the discrepancy in addition to the length of exposure to PDGF.

Our present results are inconsistent with data that showed PDGF had no detectable effect on GLUT4 translocation and stimulated glucose uptake only by activation of GLUT1 transporter in 3T3-L1 adipocytes (16–18), determined using the sucrose density gradient method. With this method, we also detected no significant increase of GLUT4MYC translocation in 3T3-L1GLUT4MYC adipocytes; there was only a significant increase in GLUT1MYC translocation after treatment with PDGF in 3T3-L1GLUT1MYC adipocytes (data not shown). By using MYC-binding assay, however, we found that PDGF can transiently trigger the translocation of GLUT4MYC transporter and glucose uptake in 3T3-L1GLUT4MYC adipocytes. This transient effect of PDGF on glucose transport may be due to transient autophosphorylation of PDGFR and transient activation of PI 3-kinase induced by PDGF (28). The time courses for PDGF-induced translocations of GLUT4MYC and GLUT1MYC showed that PDGF had a longer effect on the translocation of GLUT1 transporter (Fig. 5), which meant that there were some differences between the mechanisms by which PDGF stimulated the GLUT4 and GLUT1 redistribution. The GLUT1 transporters may have a slower endocytosis rate. Because fractionation procedures are time-consuming and relatively insensitive, it seems difficult to detect the transient GLUT4 translocation induced by PDGF by using this method. But PDGF-induced GLUT1 translocation can be detected, even using this method, for the reason mentioned above. Differences in time after PDGF treatment, detection methods of GLUT4 translocation, and the days after differentiation of adipocytes may account for the discrepancy between the results.

Evidence suggests that PI 3-kinase is both necessary and
rate experiments done in triplicate. GLUT4MYC translocation and glucose uptake in 3T3-L1 adipocytes were stimulated with 100 nM insulin, 50 ng/ml PDGF-BB, or buffer alone for 10 min at 37 °C. The 2-deoxyglucose uptake (Fig. 6, E and H). To confirm this further, we wanted to detect the expression levels of PDGF receptors on the surface of single 3T3-L1 adipocyte with immunostaining and fluorescence microscopy. However, this approach was not feasible as antibody to the extracellular domain of mouse PDGFR was not available. One or both of the above possibilities may account for the different stimulations of glucose transport induced by PDGF and insulin in 3T3-L1 adipocytes.

It was demonstrated that PDGF or EGF can activate Akt and PKCα through phosphatidylinositol 3-kinase (37–39). Subsequently, studies indicated that activation of Akt and PKCα was involved in insulin-stimulated glucose uptake and GLUT4 translocation in 3T3-L1 adipocytes (40, 41). These results also support the possibility that PDGF can stimulate glucose uptake and GLUT4 translocation through activation of Akt or PKCα in 3T3-L1 adipocytes. Growth factors, such as insulin, PDGF, and EGF, may exert effects on glucose transport in cells through a common signaling pathway.

Both insulin and PDGF can stimulate GLUT4 translocation through a wortmannin-sensitive pathway, without the involvement of insulin receptor substrate-1 (42, 43), and activate many of the same signaling cascades. Cross-talk between the signaling pathways stimulated by PDGF and insulin have been reported (44, 45). Clarifying effects of PDGF on glucose transport should lead to a better understanding of effects of insulin on glucose transport in physiological situations and in the presence of diabetes.

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**FIG. 6. Effects of wortmannin on insulin- or PDGF-stimulated GLUT4MYC translocation and glucose uptake in 3T3-L1 GLUT4MYC adipocytes.** 3T3-L1GLUT4MYC adipocytes were preincubated with buffer or 0.1 μM wortmannin for 10 min at 37 °C and then were stimulated with 100 nM insulin, 50 ng/ml PDGF-BB, or buffer alone for 10 min at 37 °C. The 2-deoxyglucose uptake (A) and GLUT4MYC translocation (B) were measured as described under “Experimental Procedures.” Values represent means ± S.E. of three separate experiments done in triplicate.
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