Role of Protein Kinase C in the Regulation of Glucose Transport in the Rat Adipose Cell

TRANSLOCATION OF GLUCOSE TRANSPORTERS WITHOUT STIMULATION OF GLUCOSE TRANSPORT ACTIVITY*

The possible role of protein kinase C in the regulation of glucose transport in the rat adipose cell has been examined. Both insulin and phorbol 12-myristate 13-acetate (PMA) stimulate 3-O-methylglucose transport in the intact cell in association with the subcellular redistribution of glucose transporters from the low density microsomes to the plasma membranes, as assessed by cytochalasin B binding. In addition, the actions of insulin and PMA on glucose transport activity and glucose transporter redistribution are additive. Furthermore, PMA accelerates insulin's stimulation of glucose transport activity, reducing the t½ from 3.2 ± 0.4 to 2.1 ± 0.2 min (means ± S.E.). However, the effect of PMA on glucose transport activity is approximately 10% of that for insulin whereas its effect on glucose transporter redistribution is approximately 50% of the insulin response. Immunoblots of the GLUT1 and GLUT4 glucose transporter isoforms in subcellular membrane fractions also demonstrate that the translocations of GLUT1 in response to PMA and insulin are of similar magnitude whereas the translocation of GLUT4 in response to insulin is markedly greater than that in response to PMA. Thus, glucose transport activity in the intact cell with PMA and insulin correlates more closely with the appearance of GLUT4 in the plasma membrane than cytochalasin B-assayable glucose transporters. Although these data do not clarify the potential role of protein kinase C in the mechanism of insulin action, they do suggest that the mechanisms through which insulin and PMA stimulate glucose transport are distinct but interactive.

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One of the major metabolic actions of insulin is to stimulate glucose transport in adipose and muscle cells (1). Insulin stimulates glucose transport in isolated rat adipose cells primarily through the translocation of glucose transporters from an intracellular pool to the plasma membrane (2, 3). Two distinct species of glucose transporters have been identified in the rat adipose cell: GLUT1, which was originally cloned from HepG2 cells (4) and rat brain (5); and GLUT4, which has recently been cloned from several rat and human tissues (6–10). The latter is primarily expressed in insulin-responsive cell types and appears to play a major role in the adipose cell's glucose transport response to insulin (11–15). However, both species are translocated in response to insulin (12, 15–17), and the signaling mechanisms that mediate these effects have not yet been defined.

Recent evidence suggests that phospholipid turnover, diacylglycerol production, and protein kinase C activation may play important roles in insulin action. Insulin has been shown to stimulate phospholipid metabolism (18–23), diacylglycerol formation (22–25), and activation of protein kinase C (26–28). As initially observed by Lee and Weinstein (29), tumor-promoting phorbol esters, which can substitute for diacylglycerol as activators of protein kinase C, elicit insulin-like effects on glucose transport activity. Subsequently, phorbol esters have been shown to mimic a variety of insulin-like metabolic actions (see Ref. 28 for a brief review). In addition, the GLUT1 glucose transporter isoform has been shown to be phosphorylated after PMA1 activation of protein kinase C in human erythrocytes, 3T3-L1 adipocytes, and mature adipose cells (30–32).

On the other hand, several other reports suggest that protein kinase C does not play a role in insulin action (33–35). Down-regulation of protein kinase C by prolonged exposure to phorbol esters does not alter insulin stimulation of glucose transport activity in L6 cells (33). Phorbol esters have been shown to antagonize insulin action on glycogen synthesis and glycogenolysis (34), and Strálfors has suggested that diacylglycerol may stimulate glucose transport activity without apparent activation of protein kinase C (35).

Recent studies in Swiss 3T3 cells (36) and rat adipose cells (37) have indicated that the stimulatory action of PMA on glucose transport activity is associated with the translocation of glucose transporters from an intracellular pool to the plasma membrane. These studies employed a d-glucose-inhibitable cytochalasin B binding assay to quantify the concentrations of glucose transporters; this assay does not distinguish between the GLUT1 and GLUT4 glucose transporter

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**To whom correspondence should be sent: EDMNS/MCNEB/NIDDK, Bldg. 10, Rm. 5N102, NIH, Bethesda, MD 20892. Tel.: 301-496-5955.**
isoforms (11, 38). In the adipose cell study, Mühbacher et al. (37) further compared the effects of PMA with those of insulin on 3-O-methylglucose transport and the subcellular distribution of cytochalasin B-associable glucose transporters in rat adipose cells. They showed that insulin and PMA induce the translocation of the same numbers of glucose transporters from the low density microsomes to the plasma membranes. By contrast, the stimulation of glucose transport activity by PMA was only a third of the magnitude of that produced by insulin. However, their results were characterized by: 1) very low concentrations of glucose transporters in their subcellular membrane fractions, generally less than half those reported from this laboratory and by other investigators (1, 39); 2) an unusually low glucose transport response to insulin, 9-fold compared with 30–40-fold reported from many laboratories (1, 39); and 3) a relatively small redistribution of glucose transporters in response to insulin (40). Furthermore, Mühbacher et al. (37) did not evaluate the possible role of multiple glucose transporter species in the apparent disparity between glucose transport activity and glucose transporter translocation, a particularly relevant issue since GLUT1 has been shown to be phosphorylated in the rat adipose cell in response to PMA in combination with insulin (91, 92) whereas GLUT4 has been shown to be the primary glucose transporter species translocated (11–15).

In this study, we have again compared the ability of PMA and insulin to stimulate 3-O-methylglucose transport and glucose transporter redistribution in rat adipose cells and have also included an immunological assessment of both GLUT1 and GLUT4. By contrast to the data of Mühbacher et al. (37), our results show that the effect of PMA on glucose transport activity is approximately 10% of that of insulin whereas its effect on glucose transporter translocation represents approximately 50% of that produced by insulin. In addition, this disparity appears to involve differential effects of PMA and insulin on the translocations of GLUT1 and GLUT4. Thus, the overall evidence presented in this study suggests that the stimulatory actions of PMA and insulin on glucose transport occur through distinct but interactive mechanisms.

**EXPERIMENTAL PROCEDURES**

**Animals and Cell Preparation**—Male rats (CD strain, Charles River Breeding Laboratories) weighing about 170–200 g were used. They were fed standard NIH chow ad libitum for at least 5 days prior to study. The rats were anesthetized with a gas mixture of 70% CO₂, 30% O₂, and kept by decapitation between 8 and 9 A.M. The epithelial fat pads were removed, minced, and digested with collagenase (type 1, Cooper Biochemical) as described previously (41).

**Incubation of Adipose Cells and Determination of Glucose Transport Activity**—All incubations were carried out at 37°C in a Krebs-Ringer bicarbonate Hepes buffer, pH 7.4, containing 10 mM sodium bicarbonate, 30 mM Hepes, 200 mM adenosine, and 1% (w/w) bovine serum albumin. Insulin-stimulated cells were incubated in the presence of 3-3H2O, [3-3H]ATP, and [3-3H]labeled sheep antimalin antibody (0.1 μCi/ml) for anti-glucose transporter (anti-GLUT1) or 125I-labeled sheep anti-mouse Fab(λ) fragments (0.2 μCi/ml) for monoclonal antibody IFS (anti-GLUT4), and washed extensively. After immunoblotting, the sheets were dried and autoradiographed for 1–7 days.

**Preparation of Subcellular Membrane Fractions**—Adipose cells obtained from 50–60 rats were incubated at 37°C in four polycarbonate containers, each containing a total volume of 40 ml (cell concentration 2 × 10⁶ cells/ml), in the presence of the indicated concentrations of insulin or PMA. To enhance cell viability in these large scale incubations, 5% (v/v) bovine serum albumin was used in Krebs-Ringer bicarbonate Hepes buffer, pH 7.4, containing 200 mM adenosine (43). At the end of the incubation period, the cells from each container were washed once with 45 ml of homogenization buffer (20 mM Tris-HCl, pH 7.4, 1 mM EDTA, 255 mM sucrose) that had been equilibrated at 18°C. Approximately 20 ml of homogenization buffer was then added, and the cells were homogenized with a 50-ml Potter-Elvehjem grinder (Thomas Scientific, 3431-E25, specific clearance of 0.15 mm).

Plasma membranes and low density microsomes were prepared by differential ultracentrifugation as described in detail previously (39). Protein was determined as described by Simpson and Sonne (44) using the Coomassie Brilliant Blue method (Bio-Rad protein assay, Bio-Rad) and crystalline bovine serum albumin (Sigma) as the standard.

**Measurement of Glucose Transporter Concentration**—The concentrations of glucose transporters in subcellular membrane fractions were assessed using a specific β-glucose-inhibitable cytochalasin B binding assay as described previously (42).

**Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis and Immunoblotting**—Plasma membrane and low density microsomal proteins (80 μg/lane) were separated on sodium dodecyl sulfate-10% polyacrylamide gels (45) and transferred to nitrocellulose paper at 500 mA for 16 h. Immunodetection of the GLUT1 and GLUT4 glucose transporter isoforms was performed using antisera 379 and monoclonal antibody IFS, respectively. Rabbit antisera 379 was raised in this laboratory against the peptide Glu-Glu-Leu-Phe-His-Pro-Leu-Gly-Ala-Asp-Ser-Gln-Val, corresponding to amino acid sequence 480–492 of the carboxy-terminal region of the rat brain glucose transporter (5). Monoclonal antibody IFS was raised by James et al. (11) against partially purified rat adipose cell low density microsomes enriched in glucose transporters. Blocking of the nitrocellulose sheets was carried out in 5% (w/v) bovine serum albumin, 10 mM Tris-HCl, pH 7.4, 0.9% NaCl for 60 min at 37°C. The nitrocellulose sheets were incubated for a further 60 min at room temperature with antisera 379 or monoclonal antibody IFS at dilutions of 1:200 and 1:1,000, respectively. The sheets were washed, incubated with 125I-protein A (0.1 μCi/ml) for antisera 379 (anti-GLUT1) or 125I-labeled sheep anti-mouse Fab(λ) fragments (0.2 μCi/ml) for monoclonal antibody IFS (anti-GLUT4), and washed extensively. After immunoblotting, the sheets were dried and autoradiographed for 1–7 days.

**Assay of Protein Kinase C Activity**—Plasma membranes and the high speed supernatant obtained after centrifugation of the low density microsomes were prepared as described previously (39), with the exception that the homogenization buffer consisted of 20 mM Tris-HCl, pH 7.5, 255 mM sucrose, 2 mM EDTA, 2 mM EGTA, 2 mM dithiothreitol, 10 μg/ml leupeptin, 1 trypsin inhibitor unit/ml aproteincase inhibitor mix, 0.1 mM phenylmethylsulfonyl fluoride, and 1% (v/v) bovine serum albumin. The membranes were solubilized for 60 min on ice in homogenization buffer containing 1% Triton X-100. Insoluble material was removed by centrifugation at 300,000 × g for 45 min. Prior to the assay, both the postmitochondrial supernatant and the solubilized plasma membranes were diluted 1:20 with homogenization buffer in the absence of Triton X-100. The protein kinase C reaction mixture contained 200 μg GS-peptide (46) in 10 mM Tris-HCl buffer, pH 7.5, with 5 mM magnesium acetate, 20 μM ATP, 0.5 mM CaCl₂, and 0.5 μCi of [3-3H]ATP. Lipids were added at a final concentration of 100 μg/ml phosphatidylinerine and 10 μg/ml 1,2-diolein. Samples were assayed for protein kinase C activity as described previously (47).

**Other Materials**—APTT and PMA were obtained from Sigma. The stock solution of PMA (1 mM) was stored at −20°C in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide did not exceed 0.1% (w/v). Dithiothreitol was from Boehringer Mannheim. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels were from Avanti Polar-Lipids ( Owners Laboratories, 37). Protein was determined as described by Simpson and Sonne (44). Crystalline bovine insulin was a gift from Dr. M. Chance, Lilly. The monoclonal antibody IFS was a gift from Dr. D. E. James, Washington University School of Medicine, St. Louis, MO 65110.

**Calculations**—All calculations were carried out on the Dartmouth Time-Shared System computer facilities. Statistical significance was tested with a paired t test, and differences were accepted as significant at the P ≤ 0.05 level.

**RESULTS**

**Time Course and Concentration Dependence of PMA Stimulation of Glucose Transport**—As illustrated in Fig. 1, both insulin and PMA stimulate 3-O-methylglucose transport in the isolated rat adipose cell. However, two important differences are observed. In insulin-stimulated cells, 3-O-methylglucose transport activity is increased 30–40-fold as compared with basal cells whereas PMA increases glucose transport activity only 3–4-fold. Second, the action of insulin is more rapid than that of PMA. The half-maximal stimulation (t₅₀)
porters—Fig. 1, transporters. As seen previously in Fig. 1, PMA stimulates in the low density microsomes (Fig. 3C), roughly half the glucose transport activity 210% of the maximum response subcellular distribution of cytochalasin B-assayable glucose combination of both on glucose transport activity and the PMA and complete stimulation within 15-20 min. Of 3-0-methylglucose transport is achieved in 3.0 min (mean the means were less than 20%.

The effects of increasing concentrations of PMA on 3-O-methylglucose transport are shown in Fig. 2. The maximal stimulatory effect of PMA is achieved at 1 μM with a half-maximal effect observed at 250 nM (mean of two experiments).

**Effect of PMA on Subcellular Distribution of Glucose Transporters**—Fig. 3 shows the effects of insulin, PMA, and a combination of both on glucose transport activity and the subcellular distribution of cytochalasin B-assayable glucose transporters. As seen previously in Fig. 1, PMA stimulates glucose transport activity ≈10% of the maximum response elicited by insulin. By contrast, PMA induces an ≈3-fold increase in the concentration of glucose transporters in the plasma membrane fraction (Fig. 3B) and an ≈40% decrease in the low density microsomes (Fig. 3C), roughly half the corresponding ≈6-fold increase and an ≈60% decrease, respectively, in response to insulin. Furthermore, when cells are first stimulated with insulin and subsequently PMA is added, a further increase in 3-O-methylglucose transport is observed above the value obtained for insulin-stimulated cells (Fig. 3A). In the presence of both insulin and PMA together, the translocation of glucose transporters from the low density microsomes to the plasma membranes is enhanced over that seen with each agent alone. The order of addition of insulin and PMA does not appear to influence these latter findings; similar results are obtained if cells are first exposed to PMA and subsequently to insulin (data not shown). When PMA is substituted by the inactive phorbol ester, 4a-phorbol, no stimulation of 3-O-methylglucose transport or alteration in the subcellular distribution of glucose transporters is observed (data not shown).

An apparent disparity is thus observed between the stimulation of glucose transport activity and translocation of glucose transporters in response to PMA compared with insulin. However, the cytochalasin B binding assay does not distinguish between GLUT4, the predominant glucose transporter isoform in the rat adipose cell, and GLUT1, the minor isoform (11, 12, 15, 38). Plasma membranes from basal and from PMA- and insulin-stimulated cells were therefore immunoblotted with the rabbit anti-GLUT1 antiseraum 379 (see "Experimental Procedures") and the monoclonal anti-GLUT4 antibody 1F8 (11). As shown in representative experiments in Fig. 4, PMA typically increases the GLUT4 signal to ≈35% of the level observed in response to insulin (2.0-fold and 5.7-fold, respectively) and the GLUT1 signal to ≈87% of the insulin-stimulated level (1.35-fold and 1.54-fold, respectively). Over a large series of 12 experiments with PMA and 10 experiments with insulin, PMA increased the GLUT4 signal 2.06 ± 0.16-fold (mean ± S.E.) whereas insulin increased the GLUT4 signal 5.98 ± 0.90-fold compared with the basal state; by contrast, PMA and insulin increased the GLUT1 signal 1.38 ± 0.11-fold and 1.68 ± 0.11-fold, respectively. In the five experiments in which the effects of PMA and insulin were examined in the same experiment, PMA increased GLUT4 2.33 ± 0.21-fold compared with an insulin effect of 6.74 ± 1.63-fold whereas PMA and insulin increased GLUT1 1.58 ± 0.22-fold and 1.79 ± 0.14-fold, respectively. Corresponding relative decreases in the GLUT4 and GLUT1 signals were observed in the low density microsomes (data not shown).

We have found the magnitude of the response to insulin to be quite variable in both the plasma membranes (3.6-13.2-fold increase) and low density microsomes (25-50% decrease) when assessed by 1F8 immunoblotting; in the present experiments, insulin induces a mean 6.0-fold increase in the plasma membranes. However, we have generally found the effects of insulin to be similar when assessed by 1F8 immunoblotting and by cytochalasin B binding (an ≈6-fold increase in the plasma membranes (Fig. 3)). The sources of this variability remain to be clarified but include such factors as varying degrees of cross-contamination among membrane fractions during the fractionation procedure and varying efficiencies of immunoblotting because of nonlinear immunoreactivity and/or film exposure.

**Effect of KCN on PMA Action**—Another instance in which an apparent disparity between glucose transport activity and glucose transporter subcellular distribution has been observed is in insulin-stimulated cells exposed to isoproterenol in the absence of adenosine. Under these conditions, the insulin-stimulated rate of 3-O-methylglucose transport is inhibited markedly without reversing the insulin-induced translocation of glucose transporters (48–50). Joost et al. (49) have further
membranes from rat adipose cells treated with insulin and membranes using the monoclonal anti-GLUT4 antibody 1F8 (11) and ultracentrifugation. Western blots were then performed on the plasma PMA. Rabbit anti-GLUT1 antiserum 379 as described under "Experimental Procedures." Plasma membranes (panel B) and low density microsomes (panel C) were then prepared and the concentrations of glucose transporters determined by specific D-glucose-inhibitable cytochalasin B binding. Values represent the means ± S.E. of three to five independent experiments. *, **, significant differences compared with the basal and insulin-stimulated values, respectively.

FIG. 4. Immunoblots of glucose transporters in plasma membranes from rat adipose cells treated with insulin and PMA. Isolated cells were incubated for 30 min with 1 μM PMA or 100 nM insulin (INS), homogenized, and fractionated by differential ultracentrifugation. Western blots were then performed on the plasma membranes using the monoclonal anti-GLUT4 antibody 1F8 (11) and rabbit anti-GLUT1 antiserum 379 as described under "Experimental Procedures." The results shown are representative of 12 experiments with PMA and 10 experiments with insulin; five experiments included both agents in the same experiment. The radioactivity incorporated into the band of molecular mass of ~45 kDa recognized by monoclonal antibody 1F8 (GLUT4) was (left to right): 551 cpm (basal), 1106 cpm (PMA), and 3156 cpm (INS). The radioactivity incorporated into the band of a molecular mass of ~43 kDa recognized by rabbit antiserum 379 (GLUT1) was (left to right): 1642 cpm (basal), 2210 cpm (PMA), and 2536 cpm (INS).

demonstrated that this inhibition of insulin-stimulated glucose transport activity in the intact cell by isoproterenol can be retained in preparations of isolated plasma membranes by the addition of KCN to the cells prior to homogenization. To determine if PMA induces a similarly inhibited form of the glucose transporter, PMA-treated cells were exposed to the absence and presence of KCN prior to subcellular fractionation. As shown in Fig. 5, KCN does not alter glucose transport activity in the intact cells (Fig. 5A) but does reverse the effect of PMA on the concentrations of glucose transporters in the plasma membranes and low density microsomes (Fig. 5, B and C, respectively). This finding contrasts to earlier observations that KCN does not affect the subcellular distribution of glucose transporters in insulin-stimulated cells (51) or insulin-stimulated cells subsequently treated with isoproterenol in the absence of adenosine (49). In addition, glucose transport measured directly in plasma membrane vesicles prepared from the PMA-treated cells in the absence and presence of KCN correlates closely with glucose transporter concentration (data not shown).

Effect of PMA on Insulin Action—In order to examine the process by which PMA and insulin might interact in regulating adipose cell glucose transport activity, the time course and reversal of insulin action on 3-O-methylglucose transport were examined in the absence and presence of PMA. As illustrated in Fig. 6A, PMA treatment reduces the time required for half-maximal stimulation by insulin by ~50%, from 3.2 ± 0.4 to 2.1 ± 0.2 min (n = 4). However, Fig. 6B illustrates that prior exposure of cells to PMA does not affect the rate of reversal of insulin-stimulated glucose transport activity as achieved using the collagenase digestion technique described by Kono et al. (52).

Effects of PMA on Protein Kinase C Activity—Fig. 7 shows the protein kinase C activities in isolated plasma membranes and the postmicrosomal supernatant following stimulation of adipose cells with PMA. Under basal conditions, the specific activity of protein kinase C in the supernatant is ~3-fold greater than that in the plasma membranes in the absence of phospholipids, but ~8-fold greater in the presence of phospholipids. This is explained by an ~3-fold stimulation of protein kinase C activity by added phospholipids in the supernatant compared with only an ~60% increase in activity in the plasma membranes. Treatment of cells with PMA induces an ~10-fold increase in protein kinase C activity in the isolated plasma membranes and a corresponding ~60% decrease in the cytosol fraction in both the absence and presence of added phospholipids.

DISCUSSION

In this study we show that PMA stimulates glucose transport activity more slowly than insulin (Fig. 1) and that this effect of PMA is relatively small, representing ~10% of the maximum response elicited by insulin (Figs. 1–3). These observations are essentially in agreement with the findings of Cherqui et al. (53) and Kirsch et al. (54) in rat adipose cells. However, the results differ from those of Farese et al. (55) who have shown that in BC3H-1 myocytes, PMA produces a greater increase in glucose transport activity than insulin. Thus, PMA may elicit different responses on glucose transport activity in different cell types.

The data presented in this study also differ from the results
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**Fig. 5.** Effect of KCN on PMA-stimulated glucose transport activity and the subcellular distribution of glucose transporters in rat adipose cells. Isolated cells were incubated at 37 °C for 30 min with 1 μM PMA and then, where indicated, for a further 5 min with 2 mM KCN. Immediately prior to homogenization, triplicate aliquots of cells were sampled for determination of 3-O-methylglucose transport (panel A) as described under "Experimental Procedures." Plasma membranes (panel B) and low density microsomes (panel C) were then prepared and the concentrations of glucose transporters determined by specific D-glucose-inhibitable cytochalasin B binding. Values represent the means of two independent experiments; the circles indicate the individual values from each experiment.

**Fig. 6.** Effect of PMA on the time course and reversal of insulin stimulation of glucose transport activity in rat adipose cells. For both experiments, isolated cells were incubated at 37 °C for 30 min in the absence (○) or presence (●) of 1 μM PMA. For panel A, insulin (10 nM) was then added and at the indicated times, 3-O-methylglucose transport was determined as described under "Experimental Procedures." The points on each curve represent the means ± S.E. of the mean values obtained from triplicate determinations in four independent experiments. For panel B, insulin was added to both basal and PMA-stimulated cells, and the incubation continued for a further 30 min. Collagenase (3 mg/ml) was then added, and at the indicated times, 3-O-methyl/glucose transport was determined. The points on each curve represent the means of the mean values obtained from triplicate determinations in two independent experiments; the variations from the means were less than 15%.

**Fig. 7.** Effect of PMA on protein kinase C activity in rat adipose cells. Isolated cells were incubated at 37 °C for 30 min in the absence or presence of 1 μM PMA. Protein kinase C activity in the postmicrosomal supernatant (cytosol) and plasma membranes was determined as described under "Experimental Procedures." The hatched bars represent enzyme activities in the presence of 0.5 mM Ca²⁺ without lipid whereas the open bars indicate the total activity in the presence of Ca²⁺ and both 100 μg/ml phosphatidylethanolamine and 10 μg/ml 1,2-dioleoyl. Values represent the means ± S.E. of three or four independent experiments.

of Mühlbacher et al. (37). Our study indicates that in rat adipose cells, PMA stimulates the translocation of cytochalasin B-assayable glucose transporters to ≈50% of that seen with insulin (Figs. 3 and 5) whereas Mühlbacher et al. (37) show that insulin and PMA induce the same degree of translocation. The explanation for this difference is not clear although it would appear to be related to the markedly diminished glucose transport response to insulin seen by these investigators, 9-fold compared with the 30–40-fold response seen here. This diminished glucose transport response would be compatible with the lower glucose transporter concentrations that these investigators observed in their subcellular membrane fractions and the similarly diminished translocation response. Thus, any differences between PMA and insulin would be more difficult to assess. Finally, although technical differences may be involved, differences in the species and/or metabolic state of the rats used could also play a role.

The evidence presented in this study suggests that insulin and PMA stimulate glucose transport activity through distinct but interactive mechanisms. The kinetics of stimulation of glucose transport activity by PMA and insulin are clearly different (Fig. 1). Furthermore, the maximum glucose transport activity and glucose transporter translocation responses elicited by PMA are less than those produced by insulin (Figs. 1 and 3). In addition, the actions of PMA and insulin on these parameters are additive (Fig. 3). Recently, Andersen et al. (56) also showed that the effects of insulin and PMA on glucose transport in rat adipose cells are additive. In general, however, other investigators have not observed this phenomenon (53, 55, 57).

A further difference between insulin- and PMA-stimulated glucose transport activity is the effect of KCN on the subcellular distribution of glucose transporters (Fig. 5). We were surprised to find that addition of KCN to PMA-stimulated cells results in the redistribution of glucose transporters from the plasma membranes to the low density microsomes. This does not occur in insulin-stimulated cells (58). Although the
reason for this effect of KCN on PMA action is unknown, it clearly distinguishes the PMA-stimulated state from the insulin-stimulated state.

Although the pathway(s) stimulated in response to insulin and PMA may differ, they appear to be interactive. PMA not only enhances maximally insulin-stimulated glucose transport activity (Fig. 3) but accelerates the rate at which the response to insulin is attained (Fig. 6). Other investigators have also shown effects of tumor-promoting phorbol esters on a variety of insulin-stimulated responses including glucose metabolism (56), DNA synthesis (59), mRNA accumulation (60), and the tyrosine kinase activity of the insulin receptor (61). Until recently, however, the effect of insulin itself on protein kinase C activity has not been clear. A number of reports have suggested that insulin activates protein kinase C (26, 27) whereas other investigators have not seen such an effect (32, 62, 63). Our own studies (28) and those of the Farese group (64) now clearly indicate that insulin significantly stimulates the activity and translocation of protein kinase C in the rat adipose cell. Furthermore, a role for protein kinase C activation in insulin’s stimulatory action on glucose transport and lipogenesis is implied from the observation that protein kinase C inhibitors diminish the stimulatory effects of insulin on these activities (65, 66).

The ratio of glucose transport activity in the intact adipose cell to cytosol/lysosome B-assayable glucose transporters present in the plasma membranes in response to PMA is markedly less than that observed in response to insulin. A potential explanation for this disparity is that insulin action on glucose transport activity might involve both the translocation of glucose transporters as well as a stimulation of their intrinsic activity once they reach the plasma membrane, as suggested by Mühlbacher et al. (37). Conversely, the action of PMA on glucose transport might be limited to stimulation of glucose transporter translocation with no accompanying increase in the intrinsic activity of the glucose transporter. The observation that PMA accelerates insulin’s stimulatory action on glucose transport activity (Fig. 6) suggests that those glucose transporters translocated in response to PMA might represent an intermediate step in insulin action. Alternatively, PMA might stimulate translocation but simultaneously inhibit glucose transporter intrinsic activity. Several studies have shown that protein kinase C activation results in the phosphorylation of the glucose transporter in several cell types (30–32). However, no evidence has been presented to indicate that phosphorylation alters the activity of the glucose transporter.

Another potential explanation, however, appears to reside in the differential effects of insulin and PMA on the translocation of the GLUT1 and GLUT4 glucose transporter isoforms in the rat adipose cell. By Western blotting of the plasma membranes (Fig. 4) and low density microsomes (data not shown), PMA appears to induce a translocation of GLUT1 which is only slightly less than that induced by insulin whereas the translocation of GLUT4 is less than 25% of that seen with insulin. Holman et al. (67), using an impermeant photolabel for cell surface glucose transporters in combination with immunoprecipitation, have similarly observed that the effect of PMA on the appearance of GLUT1 is nearly identical to that of insulin whereas the effect on GLUT4 is less than 20% of that of insulin.

Thus, the stimulation of glucose transport activity in the intact adipose cell correlates more closely with the appearance of GLUT4 than GLUT1 in the plasma membrane in response to both PMA and insulin. By contrast, the stimulation of the appearance of cytosol/lysosome B-assayable glucose transporters in the plasma membrane fraction correlates more closely with the sum of GLUT1 plus GLUT4. A corollary of this interpretation is that GLUT4 is inherently more active (higher turnover number) than GLUT1. This latter concept is supported further by measurements of glucose transport directly in the plasma membrane vesicles in which we observed a roughly 2-fold greater estimated glucose transporter intrinsic activity in vesicles from insulin-stimulated cells than from basal and PMA-treated cells (68).

In conclusion, activation of protein kinase C in response to PMA appears to trigger differentially the translocation of the GLUT1 and GLUT4 glucose transporter isoforms and thus produces disparate effects on the appearance of glucose transporters in the plasma membrane and glucose transport activity in the intact adipose cell. Nevertheless, the considerable overlap in the effects of PMA and insulin on glucose transport suggests that the distinct mechanisms of these two agents are interactive.

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