Preferential Activation of Microsomal Diacylglycerol/Protein Kinase C Signaling during Glucose Treatment (De Novo Phospholipid Synthesis) of Rat Adipocytes

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Abstract

Glucose has been reported to increase the de novo synthesis of diacylglycerol (DAG) and translocate and activate protein kinase C (PKC) in rat adipocytes. Presently, we examined the major subcellular site of PKC translocation/activation in response to glucose-induced DAG. Glucose rapidly increased DAG content and PKC enzyme activity in microsomes, but not in plasma membranes or other membranes, during a 30-min treatment of rat adipocytes. This glucose-induced increase in microsomal DAG was attended by increases in immunoreactive PKC α, β, and ϵ. Glucose-induced activation of DAG/PKC signaling in microsomes was not associated with a change in the translocation of Glut-4 transporters from microsomes to the plasma membrane, a biological response that is known to be stimulated by agonists, e.g., phorbol esters, which increase DAG/PKC signaling in plasma membranes, as well as in microsomes. In conclusion, an increase in de novo phospholipid synthesis, as occurs during glucose treatment of rat adipocytes, primarily activates DAG/PKC signaling in microsomes; moreover, this signaling response and biological consequences thereof may differ from those of agonists that primarily stimulate DAG/PKC signaling in the plasma membrane. (*J. Clin. Invest.* 1994. 93:1894–1899.) Key words: glucose • phospholipids • diacylglycerol • protein kinase C • adipocytes

Introduction

Glucose has been reported to provoke rapid increases in diacylglycerol (DAG)[1] (1, 2) and concomitantly increase the enzymatic activity (1) and/or the translocation (2) of protein kinase C (PKC) in rat adipocytes. Studies with labeled glucose in rat adipocytes have demonstrated that increases in DAG result largely, if not exclusively, from the metabolism of glucose through the pathway of de novo phospholipid (i.e., phosphatidic acid [PA]) synthesis (2). These changes in DAG and PKC have suggested that glucose can stimulate DAG/PKC signaling in rat adipocytes, and similar observations have been made in a variety of other (3, 4), but not all (e.g., see reference 5), tissues.

Glucose-induced aberrations in DAG/PKC signaling, moreover, have been postulated to play a role in the pathogenesis of diabetic complications (3, 4), and may also be involved in the pathogenesis of insulin resistance, either by persistent PKC activation and downregulation of insulin receptor tyrosine kinase activity (6) or after more chronic glucose treatment, by partial depletion of PKC-β, as shown in rat adipocytes (7).

Although glucose appears to increase DAG/PKC signaling through stimulation of de novo PA synthesis in some tissues, it seems most likely that this DAG/PKC signaling is considerably different from that resulting from agonist-stimulated hydrolysis of phospholipids through receptor-mediated activation of phospholipase C and/or D in the plasma membrane. Such a difference may be anticipated as de novo PA and consequent DAG synthesis would be expected to occur primarily in the endoplasmic reticulum (8) rather than in the plasma membrane. Presently, we evaluated this question in rat adipocytes, both with respect to the major subcellular site of glucose-stimulated DAG/PKC signaling and the PKC isoforms that are involved.

Methods

As described previously (2, 5, 7), rat adipocytes were prepared by collagenase digestion of rat epididymal fat pads obtained from 200-g male Holtzman rats fed standard laboratory chow ad lib. The cells were suspended in glucose-free Krebs–Ringer phosphate buffer (KRP) containing 1% BSA, equilibrated for 30 min at 37°C, and then treated with 0 (controls), 5, 10, or 20 mM glucose (or in a few experiments, insulin [Elanco] or tetradecanoyl phorbol-13-acetate [TPA] [Sigma Chemical Co., St. Louis, MO]) for 1–30 min as indicated in the text. (It should be noted that rat adipocytes are stable for at least 3 h in glucose-free medium, and there is little or no change in basal or insulin-stimulated glucose transport during this time period.) All treatments were added in a retrograde sequence, and the duration of incubation was kept constant, i.e., 60 min, for all samples. In experiments in which ^{32}P-labeling of immunoprecipitable MARCKS protein was evaluated, 20–30-ml adipocytes were batch incubated for 2 h in 2 vol glucose-free KRB containing 30 mM Hepes, 1% BSA, and 10 μCi of ^{32}PO$_4$ (New England Nuclear, Boston, MA). After this initial labeling period, the adipocytes were aliquoted into plastic tubes and treated with or without 10 mM glucose for 1–30 min (as above, the total incubation period was constant, i.e., in this case, 150 min, for all samples). Reactions were terminated by adding chilled KRP or KRB.

Unless indicated otherwise, adipocytes were washed and homogenized (Potter-Elvehjem homogenizer) in cold 0.25 M sucrose buffer containing 20 mM Tris/HCl (pH 7.5), 1.2 mM EGTA, 1 mM PMSF, 20 mM β-mercaptoethanol, and 20 μg/ml leupeptin (buffer I). Homogenates were centrifuged either, as described previously (2, 5, 7), to obtain cytosol and crude, Triton X-100–solubilized, total membrane fractions, or, exactly as described by Weber et al. (9), to obtain highly purified plasma membranes and microsomes and combined nuclear-mitochondrial membrane fractions.

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1. *Abbreviations used in this paper:* DAG, diacylglycerol; KRP, Krebs–Ringer phosphate buffer; PA, phosphatidic acid; PKC, protein kinase C; TPA, tissue plasminogen activator.

Immunoreactive PKC in subcellular fractions was determined by methods largely described previously (2, 5, 7), except for the use of a chemiluminescent (ECL kit; Amersham Corp., Arlington Heights, IL) detection system in some experiments, particularly those involving purified microsomes and plasma membranes. In all experiments, equal amounts of cytosolic or membrane protein from each of the treatment groups were analyzed in parallel on the same immunoblot. All anti-PKC polyclonal antisera were raised by immunization of rabbits with peptides that are contained within the variable regions (V_3) of the catalytic domains of each PKC isoform. Antisera for PKCα, γ, σ, ϵ, and ζ were obtained from Gibco (Grand Island, NY) (see references 10 and 11). Antiserum for PKCβ has been used extensively in previous studies (2, 5, 7, 11) and was obtained from Dr. John Mehegan and Dr. Bryan Roth, who have characterized this antiserum (12). As described previously (11), the epitope-recognizing specificity of each of these antisera was verified by showing that immunoreactivities of both adipocyte samples and PKC rat brain standards were no longer apparent when assays were conducted in the presence of an excess of the immunogenic peptide, which specifically blocks those antibodies that recognize the targeted amino acid sequence. As also described previously (11), (a) rat brain PKCα, β, γ, σ, ϵ, and ζ standards (and corresponding rat adipocyte PKCs) migrated on SDS-PAGE at apparent molecular masses of 80, 80, 80, 80, 90, and 75 kD, respectively; (b) using each antiserum, there was a linear relationship between observed immunoreactivity and the amount of PKC assayed, except at saturating or extremely low amounts of PKC; and (c) each of the antisera that were used did not cross-react with PKC isoforms (within the groups α, β, γ, σ, ϵ, and ζ), other than that that was targeted, as tested with recombinant PKCs that were obtained from baculovirus–insect cell and NIH/3T3 cell expression systems. DAG was measured by the method of Preiss et al. (13).

As described previously (14), membrane PKC enzyme activity was measured in vitro by the method of Chakravarthy et al. (15) as follows. Total postnuclear (i.e., post-500 $g \times$ 5 min) or purified plasma membrane or microsomal membrane fractions were examined for their ability to phosphorylate a soluble, exogenous, specific, 85-kD PKC substrate. (This 85-kD PKC substrate appears to be a MARCKS protein, as evidenced by its acidic nature and heat stability [15] and as we have reported [14] by [a] its immunoprecipitation and immunodetection by anti-MARCKS antiserum; [b] its phosphorylation by recombinant PKCα; and [c] complete inhibition of its phosphorylation by 10–100 μM [half-maximal, ~ 5 μM] PKC pseudosubstrate [Calbiochem Corp., La Jolla, CA].) The membrane fractions were washed twice, and 150 μg of membrane protein was suspended in 250 μl buffer containing 1 mM NaHCO$_3$, 50 mM Tris/HCl (pH 7.5), 5 mM MgCl$_2$, 200 μM NaVO$_4$, 200 μM NaP$_2$O$_7$, 2 mM NaF, 200 μM PMSF, and 2 μM CaCl$_2$, and then incubated for 10 min at 37°C with 20 μM [γ-^{32}P]ATP (4,000 cpm/pmol) and boiled cytosol (50 μg protein) obtained from S49T-lymphoma cells that are rich in the soluble 85-kD PKC substrate (kindly supplied by Dr. Balu R. Chakravarthy). After incubation, ice-cold EGTA solution (final concentration, 1 mM) was added to stop the reaction, membranes were removed by centrifugation at 4°C for 1 h at 100,000 g, soluble proteins were resolved by SDS-PAGE, and the ^{32}P-labeled, 85-kD PKC substrate contained therein was quantified by autoradiography and scanning laser densitometry. As reported previously (14), ^{32}P-labeling of this soluble 85-kD PKC substrate (which was added in excess) was linear with respect to time and the concentration of either membrane protein or other sources of PKC, e.g., we used column-purified, recombinant PKCα obtained from a baculovirus–insect cell expression system; no labeling of the soluble 85-kD PKC substrate was observed in the absence of membranes or other PKC sources; and there was no release of soluble 85-kD ^{32}P-labeled protein from membranes incubated in the absence of added soluble substrate. Further, the ^{32}P-labeling of the 85-kD PKC substrate is markedly stimulated by recombinant PKC, but (see reference 15) not by calcical-modulin or cAMP, and the acidic, heat-stable, 85-kD substrate is itself not PKC. It should be noted that, in the assay, the membranes were assayed in their "native" state (see reference 15), rather than after detergent solubilization and addition of exogenous phospholipids/

DAG, as frequently used with PKC assays using less specific substrates such as histone IIIs. Thus, measured PKC activity is a reflection of both membrane PKC content and endogenous PKC activators, such as membrane DAG, phospholipids, and possibly other substances.

As described previously (14), ^{32}P-labeling of MARCKS protein in intact rat adipocytes was determined by subjecting equal amounts of ^{32}P-labeled cellular protein (200–250 μg) to immunoprecipitation by incubation for 16 h at 4°C, first, with an excess (1:20 dilution) of anti-MARCKS antiserum (kindly supplied by Drs. Ivar Walaas, Otto Walaas, and Paul Greengard) obtained from rabbits inoculated with rat brain MARCKS protein (see reference 16) and, second, for 4–8 h with sheep anti–rabbit IgG antiserum (Sigma Chemical Co.). Resultant immunoprecipitates were collected by centrifugation at 100,000 g for 30 min, washed three times, resuspended in Laemmli buffer (17), boiled for 10 min, subjected to one-dimensional SDS-PAGE, and analyzed by autoradiography and scanning laser densitometry. In adipocytes, immunoprecipitable MARCKS migrates at an apparent molecular mass of 85 kD on SDS-PAGE (14).

Translocation of Glut-4 glucose transporters from low density microsomes to the plasma membrane was measured essentially as described (9, 18–20), using anti–Glut-4 antiserum obtained from East-Acres Biologicals (Southbridge, MA; see references 19 and 20).

Results

Alterations in membrane diacylglycerol content. 10 mM glucose provoked rapid 50–70% increases in microsomal DAG, which persisted throughout the 30-min treatment period (Fig. 1 and Table I). In contrast, there were no significant increases in DAG contents of the plasma membrane or nuclear–mitochondrial fraction during the 30-min period of 10 mM glucose treatment (Fig. 1 and Table I).

Alterations in PKC enzyme activity (85-kD substrate phosphorylation in vitro.) As an indicator of PKC activation, we examined the enzymatic activity of various preparations of adipocyte membranes wherein PKC activation presumably occurs. In total postnuclear membranes that were obtained from control or glucose-treated adipocytes, 10 mM glucose provoked, over a 10-min period, increases of 150±6% (mean±SE; $n = 3$ experiments; $P < 0.025$, paired t test), and these increases
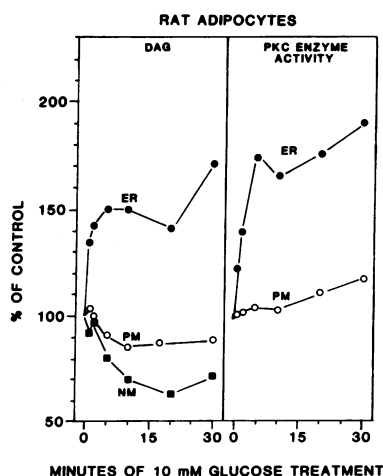
Figure 1. Time-dependent effects of glucose on diacylglycerol (*DAG*) levels and PKC enzyme activity in membrane fractions of rat adipocytes. Adipocytes were equilibrated in glucose-free KRP medium containing 1% BSA and then treated for indicated times with 10 mM glucose. Membrane fractions (*PM*, plasma membranes; *ER*, microsomes; *NM*, nuclei plus mitochondria) were purified and analyzed as described in Methods. Data are plotted as percent of the control. Changes in DAG membrane content and PKC enzyme activity (i.e., membrane-dependent 85-kD PKC substrate phosphorylation in vitro) are means of two closely agreeing experiments. Also see Table I for results from a larger number of experiments.
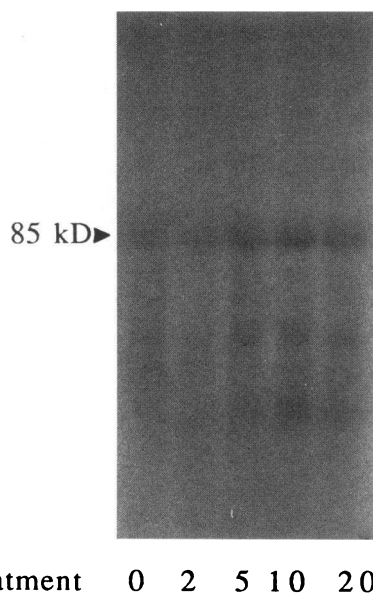
Table I. Effects of Glucose Treatment on DAG Content and PKC Enzyme Activity in Plasma Membrane and Microsomal Fractions of Rat Adipocytes

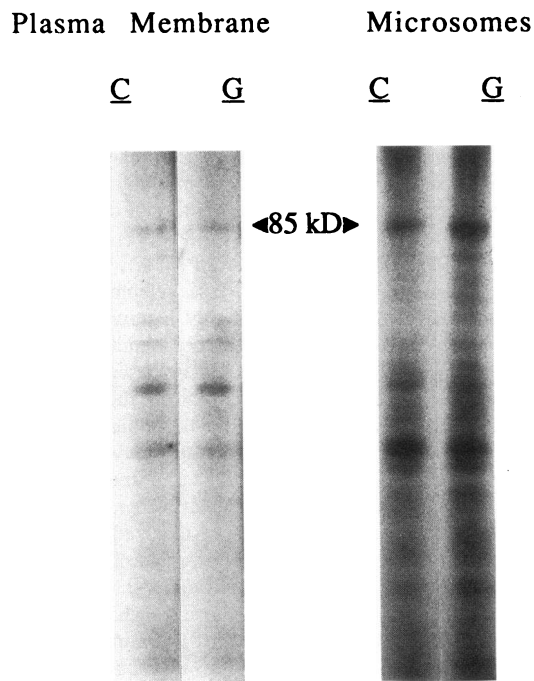| DAG content*		PKC enzyme activity*	
Plasma membrane	Microsomal fraction	Plasma membrane	Microsomal fraction
+2±9 (7)	+55±11 (7)	+22±9 (7)	+107±20 (7)
NS	$P < 0.005$	NS	$P < 0.001$

Adipocytes were equilibrated for 30 min in glucose-free KRP containing 1% BSA, and then incubated for another 10 min without (control) or with 10 mM glucose. Shown here are glucose-induced changes (relative to controls) in DAG content and PKC enzyme activity (i.e., exogenous 85-kD PKC substrate phosphorylation in vitro) in plasma membrane and microsomal fractions. Mean values±SE of (n) experiments. P was determined by paired t test. * Percent change due to 10 mM glucose treatment versus control.

appeared to be maximal or near maximal within 5 min (Fig. 2). Upon further fractionation of membranes, increases in the PKC enzyme activity of microsomal, but not plasma membrane, fractions were observed during 10 mM glucose treatment (Figs. 1 and 3, and Table I).

Translocation of PKC isoforms as determined by immunoblot analysis. We have reported previously that glucose stimulates the translocation of PKC enzyme activity and immunoreactive PKCβ from the cytosol to crude membrane fractions of rat adipocytes in a time- and dose-dependent manner (2, 5). This was presently confirmed for PKCβ in both dose-response (Fig. 4) and time course (Fig. 5) experiments, and effects of 10
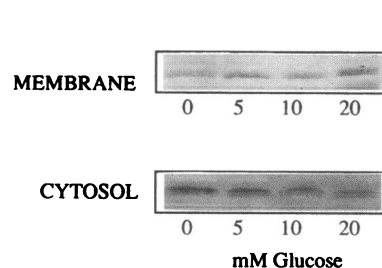
Plasma Membrane   Microsomes

C   G   C   G

◄85 kD►

Figure 3. Effects of glucose on PKC enzyme activity of plasma membranes (*left*) and microsomes (*right*) of rat adipocytes. Adipocytes were treated for 10 min without (control, C) or with (G) 10 mM glucose. Membranes were then analyzed for PKC enzyme activity in vitro as described in Fig. 2. Shown here are representative autoradiograms. Also see Table I for results from multiple experiments.

mM glucose were also compared with those of the phorbol ester, viz., 500 nM TPA (Fig. 5): as is apparent, peak increases in membrane PKCβ were frequently, although not always, observed at 5–10-min treatment with glucose or TPA, although cytosolic PKCβ continued to decrease over a 20-min period. As is also apparent, 500 nM TPA was much more potent than 10 mM glucose in stimulating the translocation of PKCβ. We also examined whether glucose provoked changes in the subcellular distribution of several other PKC isoforms that are found in rat adipocytes (11). Rat adipocytes were treated with 0 (control), 10, or 20 mM glucose for 10 min, and the cytosol and crude membrane fractions were examined for changes in their contents of various immunoreactive PKC isoforms. As shown in Fig. 6, glucose-induced changes in PKCα or ε in these crude subcellular fractions were not readily apparent, although TPA was very effective in stimulating the translocation of these isoforms from cytosol to membrane. However, as shown below, we readily detected increases in microsomal PKCα and ε in response to glucose treatment, probably reflecting low basal

85 kD►

Minutes of Treatment   0   2   5 10   20

Figure 2. Effects of glucose on PKC enzyme activity of total postnuclear membranes of rat adipocytes. Rats were treated without (control, 0) or with 10 mM glucose for the indicated times (2–20 min). Postnuclear membranes were then isolated and assayed for PKC activity, i.e., phosphorylation of soluble, exogenous 85-kD substrate (MARCKS) in vitro, as described in Methods. Shown here is a representative autoradiogram.

MEMBRANE   0   5   10   20

CYTOSOL   0   5   10   20

mM Glucose

Figure 4. Dose-related effects of glucose on translocation of PKCβ from cytosol to crude total membranes of isolated rat adipocytes. Time of treatment was 10 min. A representative immunoblot is shown here.
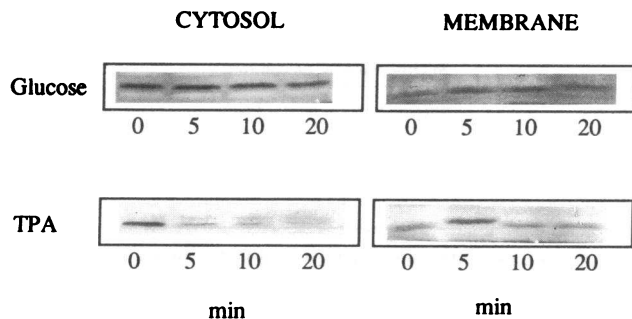
CYTOSOL   MEMBRANE

Glucose

0   5   10   20   0   5   10   20

TPA

0   5   10   20   0   5   10   20

min   min

Figure 5. Time-dependent effects of glucose (10 mM) and TPA (500 nM) on translocation of PKCβ from cytosol to crude total membranes of rat adipocytes. A representative immunoblot is shown here.

α   ε
0   5   10   20   0   5   10   20

β   ζ
0   5   10   20   0   5   10   20

Glucose Treatment (minutes)   Glucose Treatment (minutes)

Figure 7. Effects of 10 mM glucose on PKC isoforms (α, β, ε, and ζ) in microsomes of rat adipocytes. Adipocytes were incubated for 0, 5, 10, or 20 min as indicated, and microsomal membranes were isolated and immunoblotted as described in Methods. Shown here are representative immunoblots. Also see Table II for results of multiple experiments.

levels of these isoforms in microsomal membranes and ability of glucose to translocate small (relative to TPA) amounts of these isoforms. In addition, we did not observe any changes in the subcellular distribution of immunoreactive PKCδ or ζ in response to either glucose or TPA treatment (data not shown), although it should be realized that PKCδ is largely membrane associated even in the basal state (see reference 11), and PKCζ lacks a DAG/TPA binding site.

We also examined changes in immunoreactive PKCα, β, ε, and ζ in microsomal membranes in response to 10 mM glucose treatment. As is apparent in Fig. 7 and Table II, there were consistent substantial increases in microsomal PKCα, β, and ε, but only a small change in PKCζ. In plasma membranes, PKCα increased modestly, but PKCβ, ε, and ζ were unchanged or diminished slightly (Table II).

MARCKS phosphorylation in intact rat adipocytes. To verify that PKC was truly activated by glucose treatment in intact adipocytes, we studied the phosphorylation of the specific PKC substrate, MARCKS. As shown in Fig. 8, rapid increases in endogenous MARCKS phosphorylation were observed in intact adipocytes in response to 10 mM glucose treatment. The increases observed with 10 mM glucose treatment were less than those reported previously (see reference 17) with TPA treatment (i.e., two- to fourfold).

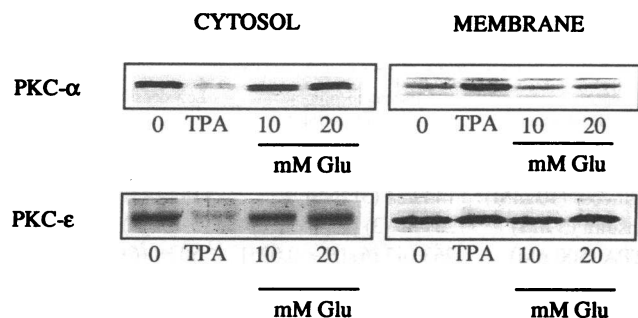*Glut-4 translocation.* In control adipocytes, as has been reported (9, 18–22), most (> 90%) immunoreactive Glut-4 was present in microsomes, with little (< 10%) in the plasma membrane. Whereas insulin or TPA treatment for 15 min provoked increases in Glut-4 translocation from low density microsomes (50% decreases) to the plasma membrane (two- to fourfold increases) (also reported previously, see references 20–23), 5 or 10 mM glucose treatment for 15 min had little or no effect on this process (Table III).

Discussion

Presently, we found that glucose-induced increases in DAG content and PKC enzyme activity in the rat adipocyte were confined to or most evident in microsomal membranes, i.e., the endoplasmic reticulum, at least initially. As may be expected, these rapid glucose-induced changes in microsomal DAG content and PKC enzyme activity were accompanied by the translocation of DAG-sensitive isoforms, PKCα, β, and ε, to microsomal membranes. In contrast to changes in microsomes, glucose did not provoke appreciable increases in plasma membrane–associated DAG, PKC enzyme activity, or immunoreactive PKCβ, ε, or ζ, although PKCα apparently increased modestly. The reason for the latter exception is presently uncertain, but the significance of this change in PKCα is diminished by the fact that total PKC enzymatic activity did not increase significantly in the plasma membrane in response to glucose treatment. Nevertheless, further studies will be needed to determine whether more prolonged glucose treatment leads to more definitive changes in plasma membrane DAG/PKC, perhaps via intracellular lipid transfer.

It should be noted that both the relatively selective increases in microsomal DAG/PKC observed during glucose treatment and the extremely low levels of immunoreactive Glut-4 in control plasma membranes indicated that there was relatively little contamination of purified plasma membranes with microsomal membranes in the presently used cellular fractionation procedure. For these reasons, the glucose-induced increase in PKCα in the plasma membrane is unlikely to be due to contamination by microsomes, although this possibility cannot be entirely excluded. It may further be noted that, although it is unlikely that microsomal membranes were contaminated with significant amounts of plasma membranes, this potential problem would only have diminished the observed effects of glucose on microsomal DAG and PKC.

The relatively selective activation of microsomal DAG/PKC signaling that occurs acutely in response to glucose treatment of rat adipocytes contrasts with rapid alterations induced

CYTOSOL   MEMBRANE

PKC-α

0   TPA   10   20   0   TPA   10   20
      mM Glu   mM Glu

PKC-ε

0   TPA   10   20   0   TPA   10   20
      mM Glu   mM Glu

Figure 6. Effects of glucose and TPA on subcellular distribution of PKCα and ε in rat adipocytes. Adipocytes were treated for 10 min with 0 (controls), 10, or 20 mM glucose, or 500 nM TPA. Cytosol and crude total membrane fractions were obtained and immunoblotted as described in Methods.
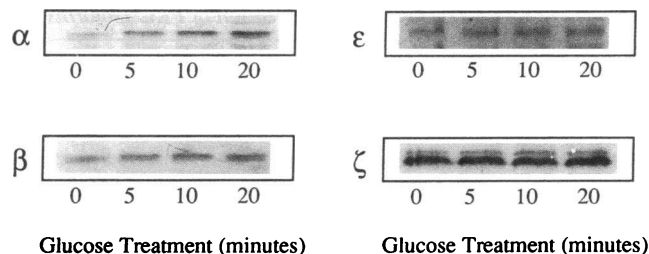
Table II. Effects of Glucose on Translocation of PKC Isoforms to Microsomes and Plasma Membranes of Rat Adipocytes

Plasma membrane PKC (% control)				Microsomal PKC (% control)			
PKCα	PKCβ	PKCε	PKCζ	PKCα	PKCβ	PKCε	PKCζ
149±4 (4)	88±7 (6)	88±18 (4)	93±14 (2)	183±15 (4)	202±33 (5)	163±18 (5)	122±0 (2)
($P < 0.005$)	NS	NS	NS	($P < 0.025$)	($P < 0.05$)	($P < 0.025$)	

Membranes were obtained after 5 or 10 min of treatment with 10 mM glucose and subjected to Western analysis for PKC isoforms. Values are mean±SE of (*n*) determinations. *P* was determined by paired *t* test.

by phorbol esters and insulin in these cells. As reported previously by Egan et al. (23) and confirmed in our laboratory using the same presently used subcellular fractionation procedures and in vitro PKC enzyme assay (14), both phorbol esters and insulin provoke rapid, sustained two- to threefold increases in PKC enzyme activity of both plasma membranes and microsomes of rat adipocytes. Furthermore, we have found that insulin rapidly increases the contents of DAG (14) and PKCα, β, and ε (unpublished) in both plasma membranes and microsomes. Thus, the failure of glucose to provoke increases in DAG and PKC enzyme activity in adipocyte plasma membranes cannot be attributed to insensitive assay methods.

The translocation of PKC isoforms to specific intracellular membranes may be expected to result in specific alterations of PKC-dependent biological processes. It was therefore of interest to find that, although phorbol esters and insulin provoked increases in the translocation of Glut-4 from microsomes to the plasma membrane, acute glucose treatment was inactive, or poorly active, in this regard. These findings indicate that (*a*) glucose-induced translocation of PKCα, β, and ε to microsomal membranes is insufficient to stimulate the translocation of Glut-4; (*b*) PKC-stimulated Glut-4 translocation must oc-

cur by means of a metabolic process(es) that is distinct from the primarily microsomal PKC activation that is provoked by glucose-induced activation of de novo synthesis of PA and DAG; and (*c*) at least initially, increases in peripheral glucose utilization that occur during hyperglycemia are more likely to result from a simple increase in "mass action" rather than an activation of the glucose transport process through a mechanism that involves Glut-4 translocation.

The fact that glucose preferentially stimulates DAG/PKC signaling in microsomes rather than in plasma membranes of rat adipocytes is important for several reasons. First, the DAG/PKC signaling and its biological consequences that occur during glucose treatment of adipocytes are, at least in some respects, likely to be considerably different from those occurring during the action of agonists that activate adipocyte plasma membrane receptors. Second, agonists or nutrients that activate or provide substrate for de novo PA synthesis may be expected to activate DAG/PKC signaling in the endoplasmic reticulum. Third, the preferential activation of PKC in the endoplasmic reticulum could have particular relevance to the pathogenesis of PKC-dependent diabetic complications that are postulated to be triggered by elevation of serum glucose (see references 3 and 4).

Finally, it should be realized that persistent PKCα activation in the plasma membrane by glucose could inhibit insulin receptor tyrosine kinase activity (see reference 6) and could thereby diminish insulin effects on various cellular processes. Also, persistent preferential activation of microsomal PKCα, β, and ε may, at least in certain tissues such as the adipocyte, lead to proteolytic degradation and diminution of the cellular content of these PKC isoforms. Accordingly, since insulin stim-
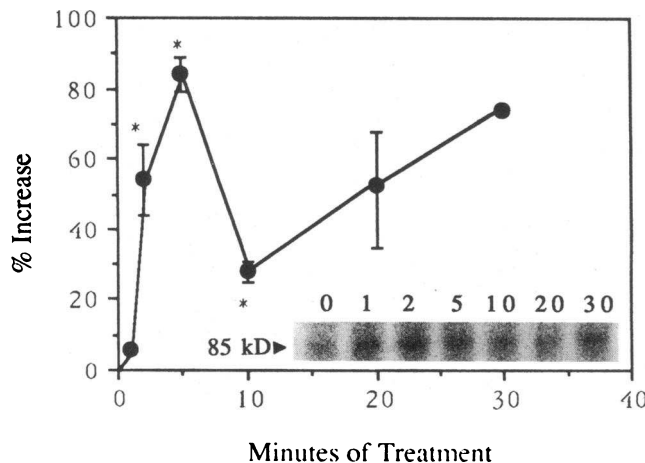

Figure 8. Effects of glucose on the phosphorylation of endogenous MARCKS in intact rat adipocytes. Adipocytes were incubated for a total of 150 min in the presence of ^{32}P, and 10 mM glucose was present during the last 30 min for the indicated times. Cell extracts were then obtained and subjected to immunoprecipitation with MARCKS antiserum, and precipitates were analyzed by SDS-PAGE and autoradiography, as described in Methods. Shown here are mean increases (±SEM) over controls in three experiments. Shown in the inset is a representative autoradiogram of labeled, immunoprecipitable, 85-kD MARCKS.

Table III. Effects of Glucose, Insulin, and Phorbol Esters (TPA) on Translocation of Glut-4 in Rat Adipocytes

Treatment	Plasma membrane (% of Control)	Low density microsomes (% of Control)
Glucose (5 mM)	134 (1)	116 (1)
Glucose (10 mM)	144±24 (3) [NS]	118±14 (3) [NS]
Insulin (3 nM)	289±5 (3) [$P < 0.001$]	55±8 (3) [$P < 0.025$]
TPA (500 nM)	263±43 (6) [$P < 0.025$]	50±9 (6) [$P < 0.005$]

Rat adipocytes were equilibrated for 30 min in glucose-free KRP containing 1% BSA, and then incubated for 15 min with or without (controls) indicated treatments. Plasma membrane and low density microsomes were then examined for immunoreactive Glut-4 levels. Mean values±SE of (*n*) experiments. *P* (in brackets) was determined by paired *t* test.

ulates the translocation of PKCα, β, and ε to both plasma membranes and microsomes in rat adipocytes (11, 14, and unpublished observations), it is possible that glucose-induced decreases in the availability of PKC (as occurring in rat adipocytes [2, 7]) may lead to decreases in insulin-induced or other agonist-induced translocation of these PKC isoforms to the plasma membrane, and possibly to other nonmicrosomal membranes. Similarly, an increase in de novo synthesis of DAG, as occurs in denervated skeletal muscle (24), could limit insulin-induced PKC translocation to critical sites and thereby diminish insulin-stimulated glucose transport in skeletal muscle. Thus, the present findings may have relevance to the pathogenesis of both "glucotoxicity" and glucose-induced insulin resistance.

Acknowledgments

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