Effect of Dexamethasone and Prednisolone on Insulin-Induced Activation of Protein Kinase C in Rat Adipocytes and Soleus Muscles

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We examined the effect of glucocorticoids on [^{3}H]2-deoxyglucose ([^{3}H]2-DOG) uptake, [^{125}I]insulin binding, tyrosine kinase activity, and protein kinase C (PKC) activity in rat adipocytes and soleus muscles. In adipocytes, insulin-stimulated [^{3}H]2-DOG uptake was decreased by prior 60-minute treatment with dexamethasone (DEX) or prednisolone (PSL), whereas [^{125}I]insulin binding, insulin (INS) receptor autophosphorylation, and tyrosine kinase activity, as measured using exogenous substrate of poly(Glu^{80}-Tyr^{20}), were not significantly changed. Cytosolic PKC activity decreased and membrane-associated PKC activity increased during a 60-minute treatment of adipocytes and soleus muscles with DEX or PSL, indicating that both DEX and PSL stimulate the translocation and activation of PKC. However, pretreatment of adipocytes and soleus muscles with glucocorticoids resulted in reduced INS-stimulated translocation of PKC from cytosol to membrane. INS-induced decreases in cytosolic PKC activity (50% ± 7% *v* 10% ± 8% and 20% ± 7%, $P < .05$ to .01, for nonpretreated [control], DEX pretreated, and PSL pretreated cells) and increases in membrane PKC (100% ± 10% *v* 50% ± 9% and 20% ± 9%, $P < .01$, for control, DEX pretreated, and PSL pretreated cells) were larger in nonpretreated adipocytes than in adipocytes pretreated with glucocorticoids. These results raise the possibility that glucocorticoids, namely, DEX and PSL, stimulate the translocation and subsequent degradative downregulation of PKC, and that this may be pertinent to their inhibitory effects on INS-stimulated glucose transport.

GLUCOCORTICOIDS have been reported to decrease insulin (INS) sensitivity and/or responsiveness in in vivo and in vitro studies.[1-4] In the rat adipocyte, for example, both long- and short-term treatments with glucocorticoids result in inhibition of INS-stimulated [^{3}H]2-deoxyglucose ([^{3}H]2-DOG) uptake.[5,6] Glucocorticoids may affect specific INS binding,[7] tyrosine kinase activity,[8,9] and/or glucose transporter changes, and levels,[10] but it remains unclear how glucocorticoids induce these exact mechanisms that underlie their inhibition of INS-stimulated hexose transport.

Recently, we reported that INS induces increases in diacylglycerol–protein kinase C (PKC) signaling in various INS-sensitive tissues,[11-14] and further found that downregulation of PKC by long-term treatment of rat adipocytes with PKC activators such as phorbol ester, INS, and glucose is associated with decreases in INS-stimulated [^{3}H]2-DOG uptake.[15] Interestingly, Kleine et al[16] have reported that dexamethasone (DEX), like phorbol esters, acutely activates (and therefore may chronically downregulate) PKC.

In this study, we examined the effect of glucocorticoids on glucose uptake, INS binding, tyrosine kinase activity, and PKC activation and downregulation in rat adipocytes and soleus muscles. Here we provide evidence that the inhibitory effects of glucocorticoids on glucose uptake may be related to the degradative downregulation of PKC in these INS-sensitive tissues.

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Submitted December 14, 1993; accepted May 12, 1994.

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0026-0495/95/4403-0004$03.00/0

MATERIALS AND METHODS

Materials

Pork INS was obtained from Novo (Copenhagen, Denmark). [γ-^{32}P]adenosine triphosphate (3,000 Ci/mmol), [1,2-^{3}H]2-DOG ([^{3}H]2-DOG), and L-[1-^{14}C]glucose (47 mCi/mmol) were purchased from New England Nuclear (Boston, MA). Phosphatidylserine (PS), diolein, histone (type III-S), phenylmethylsulfonyl fluoride, leupeptin, 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), bovine serum albumin (BSA), D-glucose, and adenosine triphosphate were purchased from Sigma Chemical (St Louis, MO). Silicone oil was obtained from Aldrich Chemical (Milwaukee, WI). All other chemicals were of reagent grade or better.

Adipocyte Experiments

Male Wistar rats weighing 150 to 200 g were fed ad libitum and killed by decapitation. Isolated adipocytes were obtained by collagenase digestion of rat epididymal fat pads[17] in Krebs-Ringer phosphate buffer (pH 7.4) containing 127 mmol/L NaCl, 12.3 mmol/L NaH_2PO_4, 5.1 mmol/L KCl, 1.3 mmol/L $MgSO_4$, 1.4 mmol/L $CaCl_2$, 3% BSA, and 2.5 mmol/L glucose. Adipocytes were washed and preincubated at 37°C in glucose-free Krebs-Ringer phosphate buffer containing 1% BSA for 30 minutes and then incubated with or without 10^{-12} to 10^{-7} mol/L DEX or 10^{-11} to 10^{-6} mol/L prednisolone (PSL), followed by incubation with INS for 30 minutes. [^{3}H]2-DOG (0.08 μCi) and unlabeled 2-DOG (0.05 mmol/L) were then added to 300 μL of a 10% (vol/vol) adipocyte suspension, and uptake of [^{3}H]2-DOG was measured over 1 minute.[18]

In PKC experiments, reactions were terminated by addition of 10 mL ice-cold buffer I (20 mmol/L Trishydrochloride, pH 7.5, 0.25 mol/L sucrose, 1.2 mmol/L EGTA, 0.1 mmol/L phenylmethylsulfonyl fluoride, 20 μg/mL leupeptin, and 20 mmol/L 20-mercaptoethanol). The adipocytes were washed twice and homogenized in buffer I. Homogenates were centrifuged at 1,000 × *g* for 2 minutes, and floating fatty materials were removed. Resultant homogenates were centrifuged at 105,000 × *g* for 60 minutes to obtain cytosol and membrane fractions as described below.

Skeletal Muscle Experiments

Soleus muscles were excised, and tension was maintained by ligatures attached to the tendon. The two soleus muscles from each rat provided one control and one stimulated sample as described previously.[12] Soleus muscles were incubated at 37°C in Erlenmeyer flasks under 95% O_2/5% CO_2 in 5 mL Krebs-Ringer bicarbonate buffer (KRBB) containing 119 mmol/L NaCl, 4.8 mmol/L KCl, 1 mmol/L NaH_2PO_4, 1.2 mmol/L $MgSO_4$, 1 mmol/L $CaCl_2$, 24 mmol/L $NaHCO_3$, 12 mmol/L HEPES (pH 7.4), 0.1% BSA, 5 mmol/L glucose, and 2 mmol/L sodium pyruvate, unless otherwise specified.

For measurements of [^{3}H]2-DOG uptake, soleus muscles were first incubated for 30 minutes in KRBB and then incubated for 30 minutes in glucose-free KRBB with or without (controls) INS, TPA, DEX, or PSL. [^{3}H]2-DOG (1 μCi), unlabeled 2-DOG (0.1 mmol/L), and L-[^{14}C]glucose (0.1 μCi) were then added and incubation was continued for 10 minutes. After incubation, tissues were removed, rapidly rinsed in isotope-free medium, blotted, weighed, homogenized in 0.5% trichloroacetic acid, and counted simultaneously for ^{14}C and ^{3}H. Corrections for [^{3}H]2-DOG in tissue samples unrelated to specific transport were determined by measurement of radioactivity of L-[^{14}C]glucose. Uptake of [^{3}H]2-DOG in soleus muscles was linear with respect to time and was inhibited by cytochalasin B.

In PKC experiments, rat soleus muscles were incubated in glucose-free KRBB and treated for 10, 30, and 60 minutes with or without DEX or PSL, and then treated for 30 minutes with insulin. In each experiment, control, INS-treated, DEX-treated, PSL-treated, DEX plus INS–treated, or PSL plus INS–treated muscles were pooled, ie, PKC enzyme activity was immediately assayed and cytosolic and membrane-associated samples were pooled at −90°C for immunoblot analysis as described below.

PKC Studies

Rat soleus muscles, adipocytes, or brain were homogenized with a polytron homogenizer in 20 mmol/L Tris hydrochloride buffer (pH 7.5) containing 0.25 mol/L sucrose, 1.2 mmol/L EGTA, 0.1 mmol/L phenylmethylsulfonyl fluoride, 20 μg/mL leupeptin, and 20 mmol/L 2-mercaptoethanol (buffer I). The homogenates of soleus muscles or adipocytes were centrifuged for 60 minutes at 105,000 × *g* to obtain cytosol and membrane fractions. After membrane fractions were resuspended in buffer I containing 5 mmol/L EGTA, 2 mmol/L EDTA, and 1% Triton X-100 for 30 minutes at 4°C, they were sonicated and then centrifuged at 105,000 × *g* to obtain solubilized membrane fractions. To measure PKC enzyme activity of rat soleus muscle, adipocytes, or brain, cytosolic or solubilized membrane fractions were diluted with 20 mmol/L Trishydrochloride buffer (pH 7.5) containing 0.5 mmol/L EGTA, 0.5 mmol/L EDTA, and 10 mmol/L 2-mercaptoethanol (buffer II). The samples were then applied to a Mono Q column (0.5 × 0.5 cm, Pharmacia HR 5/5; Pharmacia, Tokyo, Japan) that was equilibrated with buffer II and connected to a high-performance liquid chromatography system, as described previously.[11,12] PKC was eluted by application of a 20-mL linear gradient of NaCl (0 to 0.7 mol/L) in buffer II at a flow rate of 0.65 mL/min. Fractions of 1 mL were collected, and the PKC activity of each fraction was assayed by measuring the phosphorylation of histone III-S as described previously.[11] Activation of PKC in rat soleus

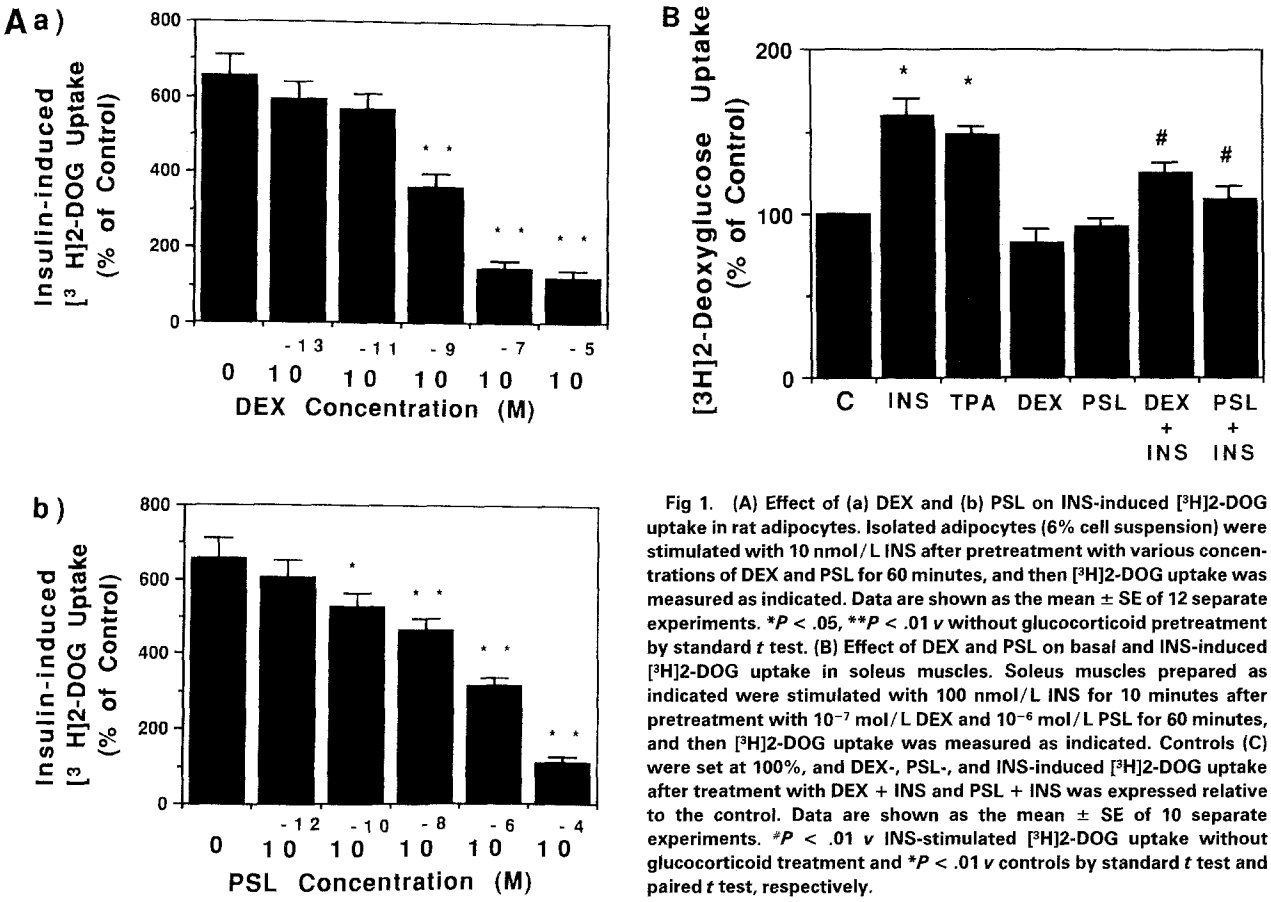

Fig 1. (A) Effect of (a) DEX and (b) PSL on INS-induced [^{3}H]2-DOG uptake in rat adipocytes. Isolated adipocytes (6% cell suspension) were stimulated with 10 nmol/L INS after pretreatment with various concentrations of DEX and PSL for 60 minutes, and then [^{3}H]2-DOG uptake was measured as indicated. Data are shown as the mean ± SE of 12 separate experiments. *P < .05, **P < .01 *v* without glucocorticoid pretreatment by standard *t* test. (B) Effect of DEX and PSL on basal and INS-induced [^{3}H]2-DOG uptake in soleus muscles. Soleus muscles prepared as indicated were stimulated with 100 nmol/L INS for 10 minutes after pretreatment with 10^{-7} mol/L DEX and 10^{-6} mol/L PSL for 60 minutes, and then [^{3}H]2-DOG uptake was measured as indicated. Controls (C) were set at 100%, and DEX-, PSL-, and INS-induced [^{3}H]2-DOG uptake after treatment with DEX + INS and PSL + INS was expressed relative to the control. Data are shown as the mean ± SE of 10 separate experiments. #P < .01 *v* INS-stimulated [^{3}H]2-DOG uptake without glucocorticoid treatment and *P < .01 *v* controls by standard *t* test and paired *t* test, respectively.

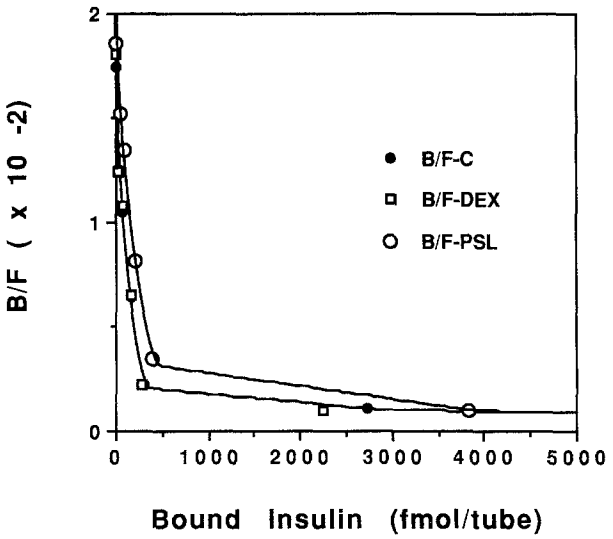

Fig 2. Effects of DEX and PSL on ^{125}I-INS–specific binding to rat adipocytes. Scatchard plots of the INS binding data from control (C) (●), DEX (□), and PSL (○) cells are as indicated in the Methods. B/F, bound/free.

muscles and adipocytes was also assayed by measuring changes in the subcellular distribution of immunoreactive PKC using methods described previously.[11,12] Partially purified rat brain PKC, which was obtained from the peak of cytosolic Mono Q column purified–PKC activity, was concentrated by a Centricon tube (Amicon, Rikaken, Tokyo, Japan), kept in 20 mmol/L Trishydochloride (pH 7.5) containing 10% glycerol at −80°C, and used for experiments of glucocorticoid effects on PKC activity in vitro. Equal amounts of cytosolic or membrane fractions were prepared as described above and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose, and incubated first with rabbit polyclonal antiserum raised to synthetic peptide specific to PKC-β and -ϵ (GIBCO, Tokyo, Japan) and second with goat antirabbit γ-globulin complexed to alkaline phosphatase. As reported,[11] this immunoblotting method detected a single major immunoreactive band that comigrated on SDS-PAGE and blotted identically with purified rat brain 80,000-M_r PKC. The intensity of immunoreactivity was scanned with a laser densitometer (Pharmacia LKB Biotechnology, Tokyo, Japan) to determine the relative value.

INS Binding Studies

Isolated fat cells were suspended in Krebs-Ringer phosphate buffer, incubated for 60 minutes with or without (control) 10^{-7} mol/L DEX or 10^{-6} mol/L PSL, and then incubated with [^{125}I]INS (2,000 Ci/mmol, Amersham, Tokyo, Japan) and unlabeled INS (1 to 1,000 nmol/L) in plastic tubes at 25°C in a shaking water bath for 60 minutes as previously described.[19] Incubations were terminated by removing 300-μL aliquots from the cell suspension and rapidly centrifuging the cells in plastic microfuge tubes to which 100 μL silicone oil had been added. The cells were then removed, and the radioactivity was determined. All studies were performed in triplicate.

Autophosphorylation, Tyrosine Kinase Activity of the INS Receptor, and GLUT4 Western Blot Analysis

For the standard phosphorylation assay, 50 μg of wheat germ agglutinin (WGA) column–purified protein[20] was preincubated at 24°C for 30 minutes with or without 10^{-7} mol/L INS. This was followed by incubation at 24°C for 10 minutes with 100 mmol/L [γ-^{32}P]adenosine triphosphate in 100 μL of 25-mmol/L HEPES buffer (pH 7.4) containing 10 mmol/L $MnCl_2$, 2 mmol/L $MgCl_2$, 1 mmol/L $NaVO_4$, and 0.1% Triton X-100. The reaction was stopped at this point by boiling for 10 minutes after addition of Laemmli buffer, and samples were analyzed by SDS-PAGE and by autoradiography for phosphorylation of 95,000-M_r protein.

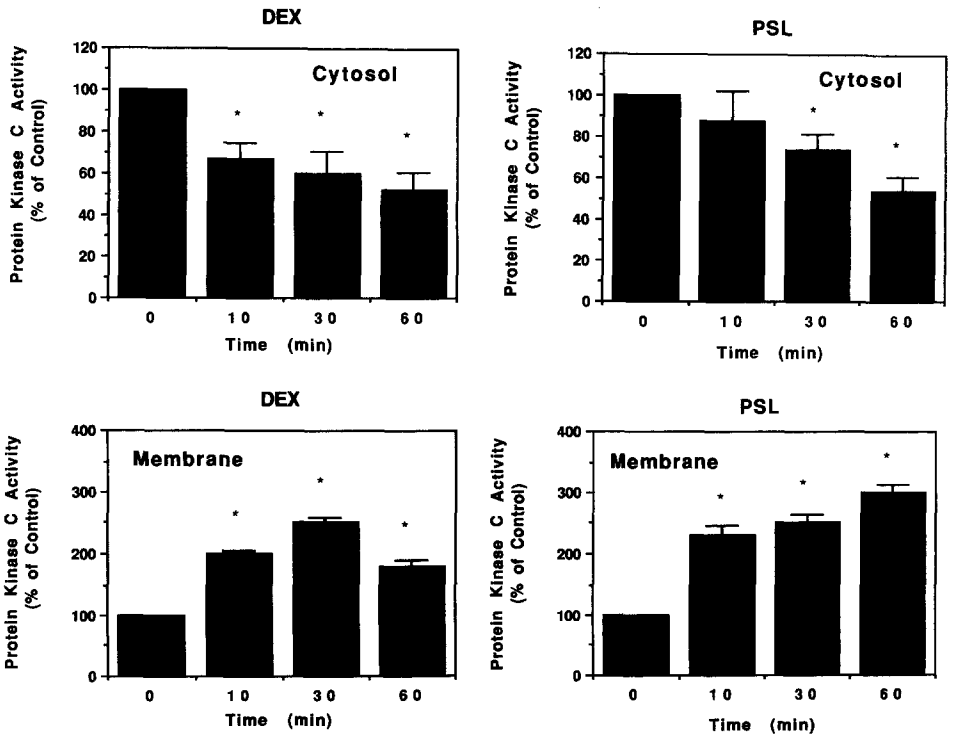

Fig 3. Glucocorticoid-induced redistribution of PKC from cytosol to membrane in rat adipocytes. Isolated adipocytes were incubated with 10^{-7} mol/L DEX and 10^{-6} mol/L PSL for 0 (control), 10, 30, and 60 minutes. At each time, cell suspensions were homogenized as indicated. Equal amounts of each cytosol and membrane protein were applied to a Mono Q column. Column-eluted PKC activity was expressed as total elutable PKC activity, which was determined by the sum of PKC activity in the presence of Ca^{2+} (0.5 mmol/L/PS) (40 μg/mL)/diolein (0.4 μg/mL) in each fraction. Control was set at 100%, and glucocorticoid treatments are expressed relative to the control. Data are plotted as the mean ± SE of five to six separate experiments. *P < .05 by paired t test.

For studies of tyrosine phosphorylation of poly(Glu^{80}-Tyr^{20}) after the 10-minute preincubation of 50 μg WGA column-purified protein, 500 mmol/L poly(Glu^{80}-Tyr^{20}) [Sigma] was added and the incubation was continued for 30 minutes at 24°C and stopped with 10% trichloroacetic acid. Precipitates were collected on Millipore filters, washed, and counted for radioactivity.

For Western blot analysis, WGA-purified INS receptors (50 to 100 μg) and membrane-associated proteins (30 μg) were subjected to SDS-PAGE on a 7.5% and 10% gel, transferred to nitrocellulose, and incubated first with monoclonal antibody to phosphotyrosine (GIBCO) and polyclonal antibody GLUT4 (kindly donated by Osamu Ezaki, National Institute of Health and Nutrition, Tokyo, Japan) and second with antimouse and antirabbit γ-globulin complexed to alkaline phosphatase to visualize the 95,000-M_r and 50,000-M_r bands, respectively.

Statistical comparisons were performed by standard Student's *t* test for planned paired comparisons where appropriate. Unless otherwise stated, all data are expressed as the mean ± SE.

RESULTS

Inhibitory Effect of DEX or PSL on [^{3}H]2-DOG Uptake

The inhibitory effects of a 60-minute treatment with DEX and PSL on INS-stimulated [^{3}H]2-DOG uptake in rat adipocytes are shown in Fig 1A (INS-stimulated [^{3}H]2-DOG uptake without glucocorticoid pretreatment *v* with treatment by 10^{-9}, 10^{-7}, and 10^{-5} mol/L DEX: 657% ± 55% *v* 466% ± 38%, 313% ± 24%, and 120% ± 18%, respectively, $^{**}P < .01$ by standard *t* test [Fig 1A, *a*]; without glucocorticoid treatment *v* with pretreatment by 10^{-10}, 10^{-8}, 10^{-6}, and 10^{-4} mol/L PSL: 657% ± 55% *v* 528% ± 34%, 466% ± 28%, 313% ± 24%, 110% ± 14%, respectively, $^{*}P < .05$, $^{**}P < .01$ by standard *t* test [Fig 1A, *b*]). Half-maximal inhibitory effects of DEX and PSL on INS-induced [^{3}H]2-DOG uptake were observed at 10^{-8} and 10^{-7} mol/L, respectively. In soleus muscles, an inhibitory effect of DEX and PSL on INS-stimulated [^{3}H]2-DOG uptake was also observed. Pretreatment with 10^{-7} mol/L DEX and 10^{-6} mol/L PSL for 60 minutes resulted in 22% and 31% inhibition, respectively (INS-stimulated [^{3}H]2-DOG uptake without glucocorticoid pretreatment *v* with 10^{-7} mol/L DEX [DEX + INS] and 10^{-6} mol/L PSL [PSL + INS] pretreatment: 160% ± 10% *v* 125% ± 7% and 110% ± 8%, respectively, $^{\#}P < .01$ by standard *t* test *v* INS, $^{*}P < .01$ by paired *t* test *v* control (Fig 1B).

Effect of PSL and DEX on INS Binding to Rat Adipocytes

DEX and PSL had no significant effect on the ability of adipocytes to bind INS (Fig 2). Cells pretreated with PSL bound 2.36% ± 0.10% (mean ± SE) of ^{125}I-insulin per tube (260 μL of a 10% adipocyte suspension). Cells treated with DEX bound 2.06% ± 0.09% (mean ± SE). There were no significant differences between cells treated without (controls: 2.14% ± 0.08%, mean ± SE) and with PSL or DEX. However, INS binding to cells treated with PSL was increased as compared with cells treated with DEX ($P < .05$), as previously reported.[5]

Glucocorticoid-Induced Redistribution of PKC From Cytosol to Membrane

As shown in Fig 3, the cytosolic PKC activity of adipocytes decreased to 52% of control values during a 60-minute treatment with 10^{-7} mol/L DEX. On the other hand, membrane-associated PKC activity increased to 250% of control values at 30 minutes, and then gradually decreased to 180% of control values after a 60-minute treatment with 10^{-7} mol/L DEX. PSL also stimulated the translocation of PKC from cytosol to membrane in adipocytes. Cytosolic PKC enzyme activity decreased to 54% of control values after 60 minutes, and membrane-associated PKC enzyme activity gradually increased to 300% for 60 minutes during treatment with 10^{-6} mol/L PSL. These results indicate that DEX and PSL translocate (activate) PKC from cytosol to membrane in adipocytes.

In the case of soleus muscle, cytosolic PKC enzyme activity also decreased to 60% of control values after 30 minutes, and to 50% of control values after 60 minutes. Membrane-associated PKC activity increased to 251% of control values after 10 minutes, 212% after 30 minutes, and

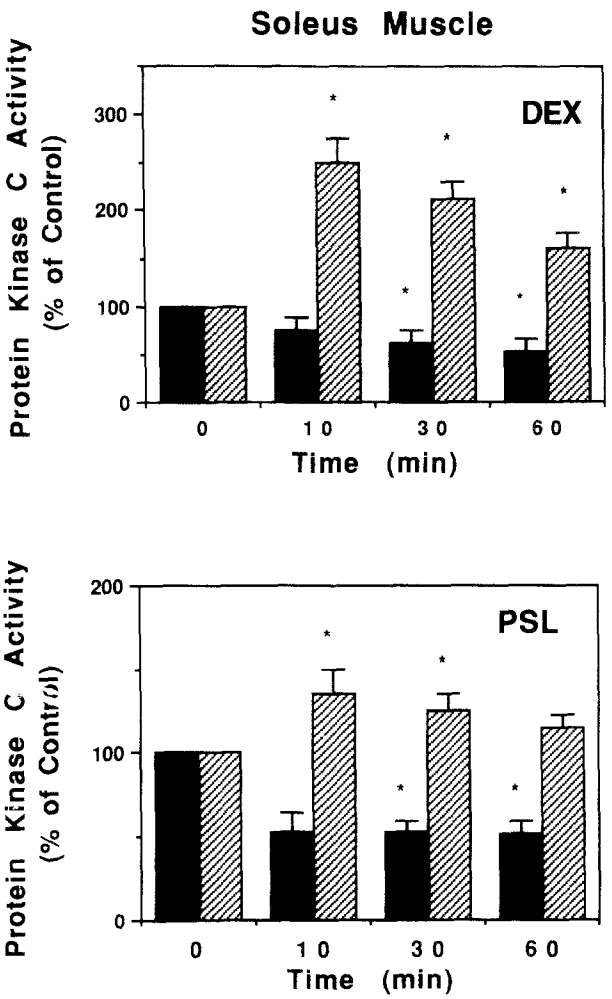

Fig 4. Glucocorticoid-induced redistribution of PKC from cytosol to membrane in soleus muscle. Soleus muscles were incubated with 10^{-7} mol/L DEX and 10^{-6} mol/L PSL for 0 (control), 10, 30, and 60 minutes. At each time, muscles were homogenized as indicated. PKC activities were measured as indicated in Fig 3. Control was set at 100%, and glucocorticoid treatments are expressed relative to the control. Data are plotted as the mean ± SE of three PSL to five DEX separate experiments. $^{*}P < .05$ by paired *t* test.

160% after 60 minutes of treatment with 10^{-6} mol/L DEX. PSL (10^{-7} mol/L) also stimulated the translocation of PKC activity from cytosol to membrane. Cytosolic enzyme activity decreased to 52% to 53% after 10, 30, or 60 minutes, and membrane-associated PKC activity increased to 135% of control values at 10 minutes, 125% at 30 minutes, and 115% at 60 minutes, respectively, during treatment with 10^{-7} mol/L PSL (Fig 4).

Redistribution of Immunoreactive PKC in Adipocytes During Treatment With Glucocorticoids

Cytosolic PKC-β and PKC-ε immunoreactivity decreased and membrane-associated PKC-β and PKC-ε increased during treatment of adipocytes with 10^{-7} mol/L DEX and 10^{-6} mol/L PSL for 60 minutes (Fig 5). Laser densitometric scanning revealed decreased cytosolic and increased membrane-associated immunoreactivity during treatment with DEX and PSL (cytosolic PKC-β immunoreactivity: control 100%, *DEX 10 minutes 78% ± 6%, *DEX 30 minutes 61% ± 7% *DEX 60 minutes 57% ± 10%, *PSL 10 minutes 91% ± 7%, *PSL 30 minutes 59% ± 8%, *PSL 60 minutes 56% ± 5%; membrane-associated PKC-β immunoreactivity: control 100%, *DEX 10 minutes 151% ± 11%, *DEX 30 minutes 249% ± 14%, DEX 60 minutes 153% ± 13%, PSL 10 minutes 146% ± 16%, *PSL 30 minutes 157% ± 18%, *PSL 60 minutes 257% ± 12%, mean ± SE, *$P < .05$ to .01).

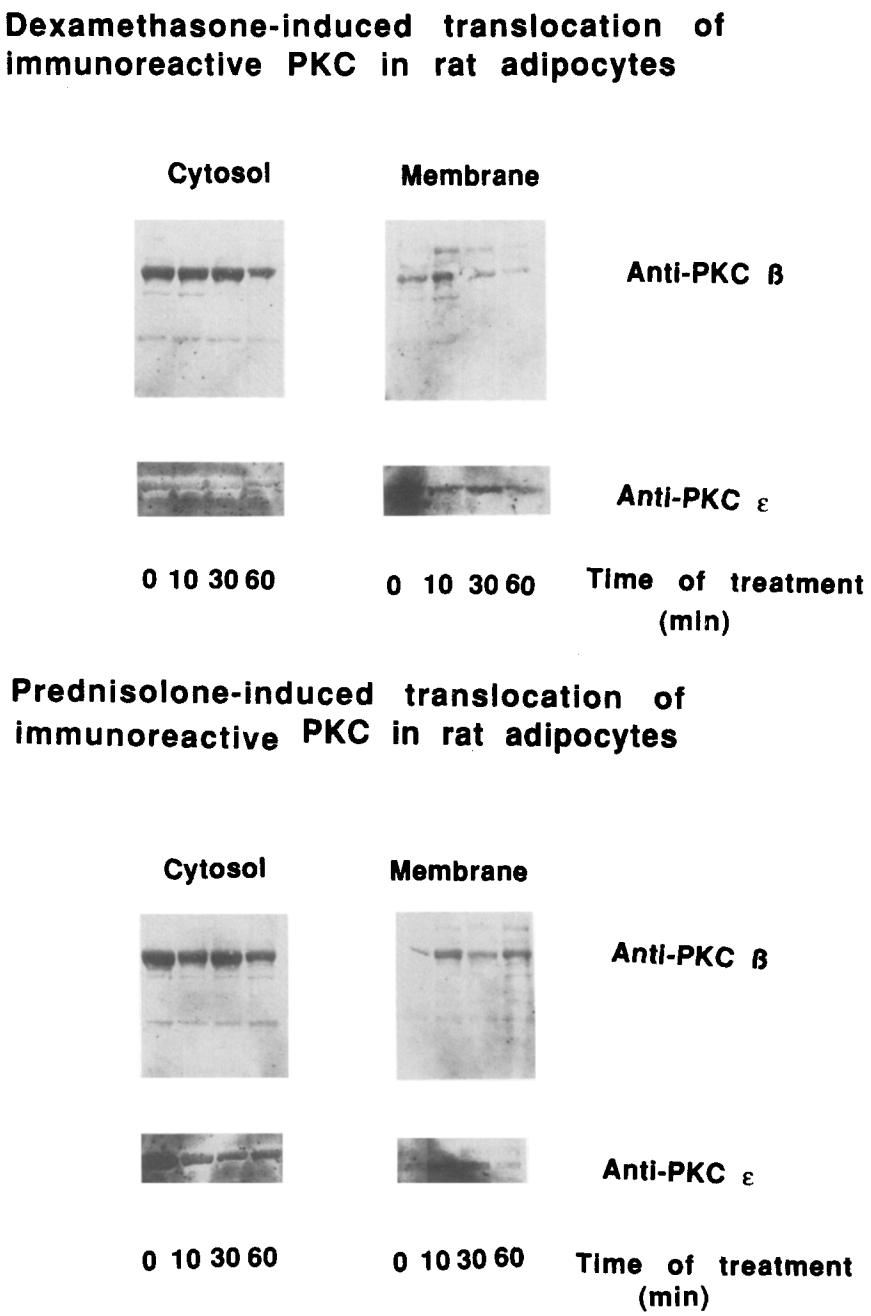

Fig 5. Immunoblot analysis of PKC-β and -ε during treatment with glucocorticoids for 60 minutes in adipocytes. Equal amounts of cytosolic and membrane protein were subjected to SDS-PAGE, transferred to nitrocellulose, and subjected first to immunologic detection with PKC-β and -ε antibodies and second to antibody coupled to alkaline phosphatase. Similar results were observed in at least five other experiments for each glucocorticoid.

Effect of DEX and PSL Pretreatment on INS-Induced Translocation of PKC in Adipocytes and Soleus Muscles

INS-induced translocation of PKC activity from cytosol to membrane was stoichiometric over a period of 10 minutes, as indicated by a 50% decrease in cytosolic and a 100% increase in membrane PKC (note that in control tissue, the ratio of cytosolic protein to membrane protein was 2:1). After pretreatment of adipocytes with DEX and PSL for 60 minutes, INS treatment resulted in only 10% decreases in cytosol PKC and 50% and 20% increases in membrane PKC, respectively (cytosolic PKC activity: control 100%, INS 10 minutes 50% ± 7%, DEX 60 minutes + INS 10 minutes 90% ± 8%, PSL 60 minutes + INS 10 minutes 80% ± 7%, DEX 60 minutes 52% ± 9%, PSL 60 minutes 54% ± 7%, $^{*}P < .01$ by paired t test, INS v control, $^{\#}P < .05$ to .01 by standard t test, DEX + INS v INS, PSL + INS v INS; membrane-associated PKC activity: control 100%, INS 10 minutes 200% ± 10%, DEX 60 minutes + INS 10 minutes 150% ± 9%, PSL 60 minutes + INS 10 minutes 120% ± 7%, DEX 60 minutes 180% ± 9%, PSL 60 minutes 300% ± 14%, $^{*}P < .01$ by paired t test, INS v control, $^{\#}P < .01$ by standard t test, DEX + INS or PSL + INS v INS). These results indicate that DEX and PSL suppress INS-induced translocation of PKC, as compared with INS effects in non–glucocorticoid-treated cells (Fig 6A). Figure 6B shows similar results in soleus muscle, ie, INS-induced decreases in cytosolic PKC activity (46% ± 8% v 21% ± 8% and 17% ± 10%, mean ± SE, for control (INS), DEX + INS, and PSL + INS muscles, respectively, $^{\#}P < .05$ by standard t test, INS v DEX + INS or PSL + INS, $^{*}P < .01$ by paired t test, control (C v INS) and increases in membrane PKC (135% ± 11% v 85% ± 13% and 60% ± 14%, mean ± SE, for INS, DEX + INS, PSL + INS, respectively, $^{\#}P < .02$ to .01 by standard t test, INS v DEX + INS or PSL + INS, $^{*}P < .01$ by paired t test, control v INS) were larger in nonpretreated soleus muscles than in soleus muscles pretreated with DEX and PSL for 60 minutes, respectively.

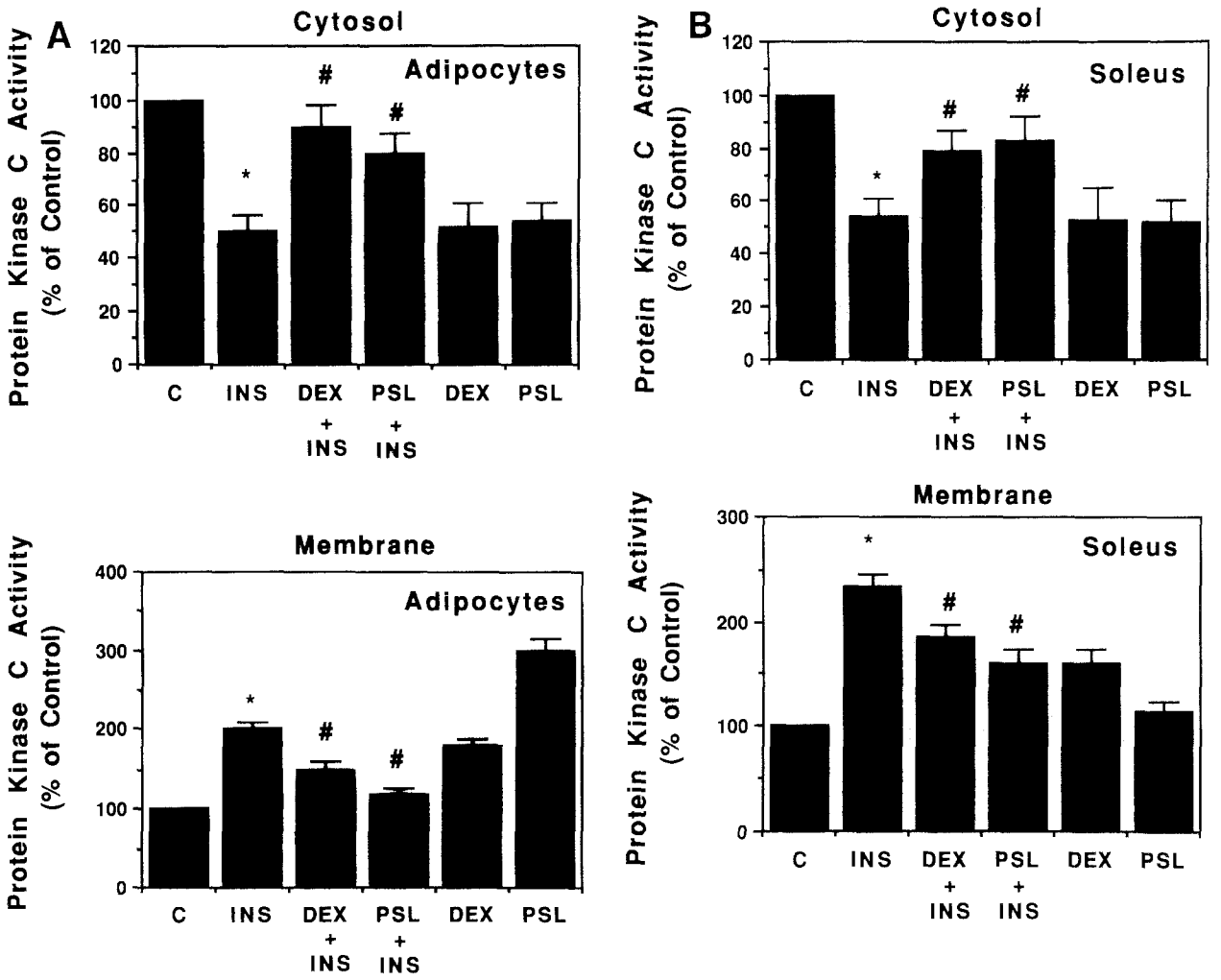

Fig 6. INS-induced activation of PKC activity from Mono Q column–eluted total PKC activities in (A) adipocytes and (B) soleus muscles with or without pretreatment with glucocorticoids. Controls were set at 100%, and INS (INS treatment for 10 minutes), DEX + INS (INS treatment for 10 minutes after pretreatment with 10^{-7} mol/L DEX for 60 minutes), and PSL + INS (INS treatment for 10 minutes after treatment with 10^{-6} mol/L PSL for 60 minutes) are expressed relative to the control. Data are plotted as the mean of four separate experiments. $^{*}P < .05$ by paired t test.

Effect of DEX and PSL on INS Receptor Tyrosine Kinase Activity and Autophosphorylation

Pretreatment of adipocytes with DEX for 60 minutes resulted in no stimulatory effect, whereas pretreatment with PSL resulted in a stimulatory effect (*$P < .05$ by standard t test, PSL + INS v INS) on insulin receptor tyrosine kinase activity using the exogenous substrate poly-[Glu^{80}-Tyr^{20}] (Fig 7A). Autophosphorylation of INS receptor β-subunit was not significantly increased by DEX and PSL treatment (Fig 7B), nor was phosphotyrosine immunoreactivity as measured by immunoblot analysis (data not shown).

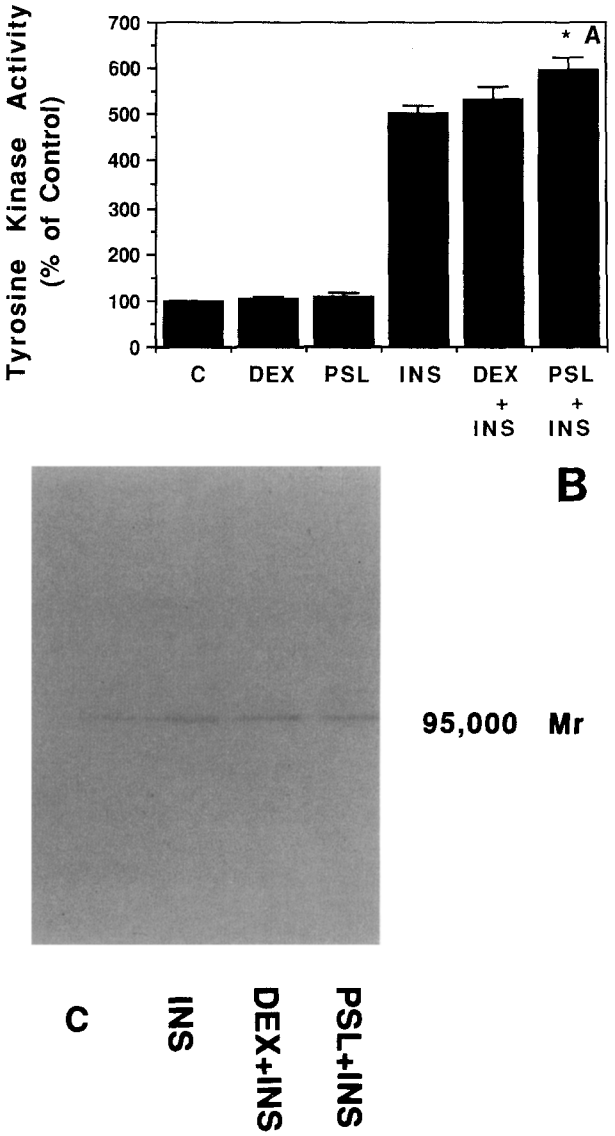

Fig 7. INS receptor tyrosine kinase activity during pretreatment with glucocorticoids in rat adipocytes. Tyrosine kinase activity was measured by (A) exogenous substrate poly[Glu^{80}-Tyr^{20}] and (B) autophosphorylation of INS receptor using WGA column as indicated. Control (C), DEX alone (DEX), PSL alone (PSL), INS-stimulated (INS), INS-stimulated pretreated with DEX for 60 minutes (DEX + INS), and INS-stimulated pretreated with PSL for 60 minutes (PSL + INS). Data are plotted as the mean ± SE of three separate experiments. *$P < .05$ by paired t test.

Effect of DEX, PSL, PS/Diolein, and Ca^{2+} on Enzymatic Activity of Partially Purified Rat Brain PKC

As shown in Fig 8, partially purified PKC from rat brain cytosol was activated by DEX (most effectively in the presence of 0.01 mmol/L Ca^{2+}), by PS and 1,2-diolein (most effective in the presence of 0.5 mmol/L Ca^{2+}), and by PSL (most effective in the presence of 0.1 mmol/L Ca^{2+}). Thus, each PKC activator had a different Ca^{2+} dependency.

Glucocorticoid-Induced Changes of GLUT4 Immunoreactivity in Rat Adipocytes

As shown in Fig 9, membrane-associated GLUT4 immunoreactivity was not changed during treatment with 10^{-7} mol/L DEX in adipocytes.

DISCUSSION

The diabetogenic effect of glucocorticoid hormones commonly observed in patients treated with these agents or suffering from endogenous glucocorticoid excess results from hepatic and peripheral INS resistance.[21] In humans, chronic and acute exposure to an excess of glucocorticoids results in a decrease in maximal INS-stimulated glucose utilization,[22,23] suggesting a postbinding defect in peripheral INS action.

In a previous study on INS binding and glucose uptake in rat adipocytes, DEX and PSL administration in vivo resulted in decreased and increased INS binding, respectively, and there was no effect of glucocorticoids on INS binding or glucose uptake in vitro (at least 2-hour incubation).[5] On the other hand, DEX acutely inhibited INS-stimulated 3-*O*-methyl glucose transport and changed [^{3}H]cytochalasin B binding without affecting INS binding, indicating that glucocorticoids altered the distribution of glucose transporters between the plasma membranes and the low-density microsomal fraction.[6]

Karasik and Kahn examined DEX-induced changes in INS receptor autophosphorylation and kinase activity of rat hepatocytes and indicated that glucocorticoids modulate INS receptor kinase activity but that the nature of their effect depends on other factors, including the dietary state of the animal, and suggested that postreceptor changes account for a major component of glucocorticoid-induced INS resistance.[24] In our experiments as indicated in Fig 7, tyrosine kinase activity using exogenous substrate poly[Glu^{80}-Tyr^{20}] in PSL-pretreated adipocytes was increased as compared with nontreated adipocytes prepared from fed rats. However, this result could not explain the glucocorticoid-induced decrease of glucose uptake. Recently, it has been reported that activation of PKC caused an inhibition of INS receptor kinase in rat adipocytes.[25] If downregulation of PKC in the cytosolic fraction occurs, INS receptor tyrosine kinase activity will probably be increased.

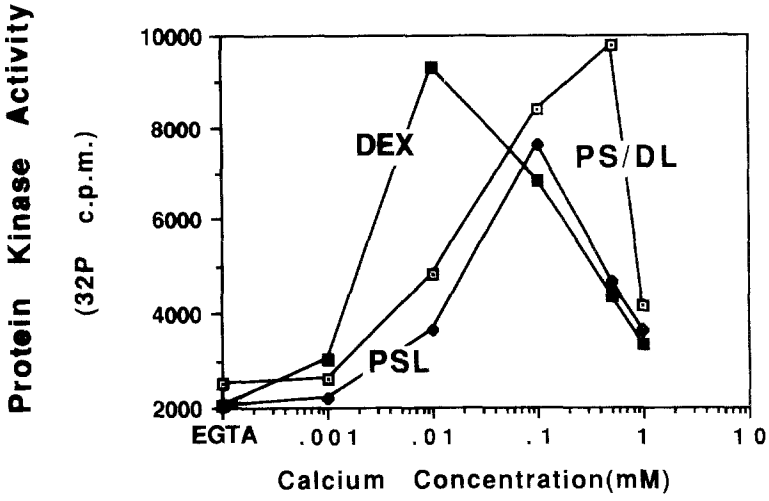

Fig 8. Ca^{2+}-dependence of the ability of DEX, PSL, and PS/diolein (PS/DL) to stimulate partially purified PKC from rat brain. One milligram of protein was applied to a Mono Q column connected to a high-performance liquid chromatography system as indicated. Partially purified PKC (specific activity, 2,000 to 3,000 pmol/min/mg protein) was assayed by histone phosphorylation activity in the presence of 10^{-7} mol/L DEX, 10^{-6} mol/L PSL, or PS/DL at various concentrations of Ca^{2+}. Data are plotted as the mean of three values in three separate experiments.

Garvey et al[10] have demonstrated that pretreatment with DEX for 15 to 180 minutes resulted in a time-dependent decrease of INS-stimulated 2-DOG uptake in adipocytes and suppression of the translocation of intracellular glucose transporter to the cell surface, and that with more chronic exposure the total number of cellular transporters decreased. They explained that INS resistance after exposure to glucocorticoids is dependent on impairment of the glucose transporter system. If glucocorticoids stimulate PKC activity, glucocorticoids alone may stimulate glucose uptake, as does phorbol ester, in insulin-sensitive tissue. However, glucocorticoids did not stimulate [^{3}H]2-DOG uptake, unlike phorbol ester, which stimulates glucose transporter translocation from low-density microsome to plasma membrane.[26] Thus, we suggest that glucocorticoids per se suppressed the translocation of glucose transporter to the cell surface, as indicated by Garvey et al,[10] despite glucocorticoid-stimulated PKC activation and decrease of cytosolic PKC content.

The present experiments were undertaken to test the hypothesis that glucocorticoid-induced peripheral INS resistance is due to a postbinding defect probably involving PKC. Indeed, we could discern no inhibitory effects of DEX or PSL on INS binding to the receptor or on autophosphorylation of the INS receptor, except for INS receptor tyrosine kinase activity using exogenous substrate poly[Glu^{80}-Tyr^{20}]. DEX and PSL were found to provoke the translocation of PKC enzyme activity and PKC-β and -ε immunoreactivity from the cytosol to membrane fractions in both adipocytes and soleus muscles. Also, in a previous study DEX activated PKC in T51B rat liver cells,[16] and we found that there was no effect of glucocorticoids on [^{3}H]phorbol 12,13-dibutyrate binding activity to PKC and that [^{3}H]DEX directly bound to purified PKC (unpublished results, November 1993). Moreover, we found in the present study that DEX and PS directly activated rat brain purified PKC when assayed in the presence of Ca^{2+}/PS/diolein.

Some investigators have reported that the mRNA and protein for the GLUT1 glucose transporter, which is coexpressed with GLUT4 in adipocytes, is decreased.[27,28] In contrast, it has also been reported recently that the GLUT4 isoform in rat skeletal muscle is not decreased by DEX.[29] In our experiments, GLUT4 immunoreactivity of adipocyte membrane was not changed by DEX and PSL for 60 minutes, as indicated in Fig 9.

Our findings indicate that DEX and PSL directly activate PKC in vitro and translocate PKC-β and PKC-ε in adipocytes and soleus muscles. Concomitant with translocative activation of PKC, DEX and PSL treatment resulted in decreases in cytosolic PKC and diminished effects of INS on PKC translocation. Since INS-induced PKC translocation has been implicated in the stimulation of hexose transport, it is possible that glucocorticoids inhibit the INS effects on hexose uptake by acutely downregulating PKC, which is identical with a decrease of cytosolic PKC. Further studies will be required to test this hypothesis.

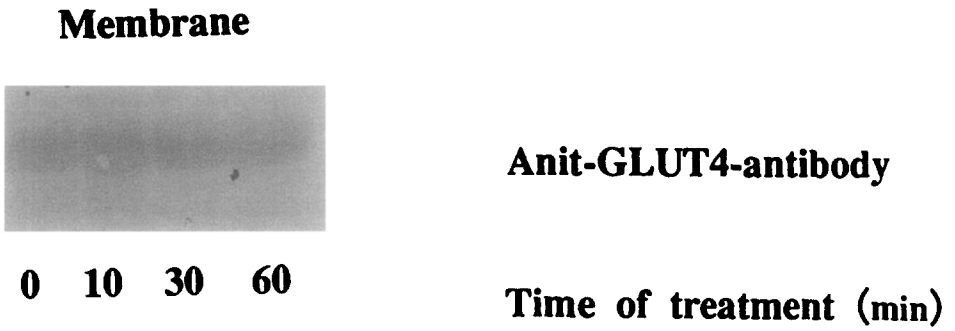

Fig 9. Immunoblot analysis of GLUT4 in rat adipocytes stimulated with 10^{-7} mol/L DEX. Isolated adipocytes were stimulated with 10^{-7} mol/L DEX, washed, homogenized, and centrifuged, and then the membrane-associated fraction was subjected to SDS-PAGE, transferred, and incubated with GLUT4 antibody coupled to alkaline phosphatase. Similar results were observed in three separate experiments.

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