Thiazolidinedione Treatment Enhances Insulin Effects on Protein Kinase C-\(\zeta\)/Activation and Glucose Transport in Adipocytes of Nondiabetic and Goto-Kakizaki Type II Diabetic Rats*

We evaluated effects of the thiazolidinedione, rosiglitazone, on insulin-induced activation of protein kinase C (PKC-\(\zeta\))/and glucose transport in adipocytes of Goto-Kakizaki (GK)-diabetic and nondiabetic rats. Insulin effects on PKC-\(\zeta\)/and 2-deoxyglucose uptake were diminished by approximately 50% in GK adipocytes, as compared with control adipocytes. This defect in insulin-stimulated PKC-\(\zeta\)/activation was associated with diminished activation of IRS-1-dependent phosphatidylinositol (PI) 3-kinase, and was accompanied by diminished phosphorylation of threonine 410 in the activation loop of PKC-\(\zeta\)/; in contrast, protein kinase B (PKB) activation and phosphorylation were not significantly altered. Rosiglitazone completely reversed defects in insulin-stimulated 2-deoxyglucose uptake, PKC-\(\zeta\)/enzyme activity and PKC-\(\zeta\)/threonine 410 phosphorylation, but had no effect on PI 3-kinase activation or PKB activation/phosphorylation in GK adipocytes. Similarly, in adipocytes of nondiabetic rats, rosiglitazone provoked increases in insulin-stimulated 2-deoxyglucose uptake, PKC-\(\zeta\)/enzyme activity and phosphorylation of both threonine 410 activation loop and threonine 560 auto-phosphorylation sites in PKC-\(\zeta\)/, but had no effect on PI 3-kinase activation or PKB activation/phosphorylation. Our findings suggest that (a) decreased effects of insulin on glucose transport in adipocytes of GK-diabetic rats are due at least in part to diminished phosphorylation/activation of PKC-\(\zeta\)/, and (b) thiazolidinediones enhance glucose transport responses to insulin in adipocytes of both diabetic and nondiabetic rats through increases in phosphorylation/activation of PKC-\(\zeta\)/.

Thiazolidinediones (TZDs), as activators of peroxisome proliferator-activated receptor-\(\gamma\), serve as clinically important insulin-sensitizing agents and improve overall glucose homeostasis in type II diabetes mellitus (1). Improvement in glucose homeostasis following TZD treatment is primarily due to enhanced effects of insulin on glucose transport and subsequent storage in skeletal muscle (2), and, to a lesser extent, in adipose tissue. Despite the widespread use and effectiveness of TZDs in treating type II diabetes mellitus, the underlying mechanism(s) of action of TZD remains uncertain. Whereas TZDs may provoke increases in the levels of GLUT4 (3) or GLUT1 (4) glucose transporters, TZD treatment can also enhance insulin-stimulated GLUT4 translocation to the plasma membrane or glucose transport in the absence of changes in the level of glucose transporters (5, 6). With respect to signaling factors that insulin uses to stimulate GLUT4 translocation and subsequent glucose transport, phosphatidylinositol (PI) 3-kinase is now generally recognized to be a key factor (7). In this regard, although TZDs have been reported to increase PI 3-kinase activity or reverse defects in insulin-induced activation of PI 3-kinase in certain situations (e.g. see Ref. 8), alterations in PI 3-kinase have not been observed (9) or reported in many studies.

Presently, to gain further insight into the mechanism of action of TZDs, we examined the potential role of two protein kinases that are postulated to function distally to PI 3-kinase during insulin regulation of GLUT4 translocation and glucose transport, namely protein kinase C-\(\zeta\)/ (PKC-\(\zeta\)/) (10–14) and protein kinase B (PKB or Akt) (15–18). For this purpose, we examined the effects of TZD treatment on insulin-induced activation of these kinases in adipocytes of both nondiabetic rats and non-obese type II diabetic Goto-Kakizaki (GK) rats. In the GK-diabetic rat (originally derived by repeated in-breeding of glucose-intolerant Wistar rats), plasma insulin levels are initially low (19), but, as plasma glucose levels rise, plasma insulin levels increase (20, 21), presumably compensatorily, and may be mildly elevated (in an absolute sense, but still relatively low for the degree of hyperglycemia) for a limited time (22). In conjunction with hyperglycemia and possibly modest hyperinsulinemia, an apparently secondary form of insulin resistance occurs, as evidenced by defects in (a) both hepatic glucose output and peripheral glucose disposal in muscle (20, 21) and adipose (20) tissues during euglycemic-hyperinsulinemic clamp studies, and (b) GLUT4 translocation and/or glucose transport in isolated adipocytes (23) and skeletal muscle preparations (24, 25). In association with decreases in glucose transport, impaired activation of IRS-1-dependent PI 3-kinase by insulin has also been observed in the slow twitch soleus muscle, but not in the fast twitch extensor digitorum longus muscle, of the GK rat (25). The activation of PKB by insulin has been reported to be impaired in both of these muscles in GK rats, but the reversal of these defects in PKB activation and glucose trans-
port by blood sugar normalization (by prolonged treatment with phlorizin) did not appear to be explained by alterations in PI 3-kinase activation (25). As reported here, we found that, in conjunction with defects in insulin-stimulated glucose transport and PI 3-kinase activation, there are defects in the phosphorylation and enzymatic activation of PKC-ζ/λ in adipocytes isolated from GK rats. Of further interest, in vivo treatment with the TZD rosiglitazone reversed the defects in insulin-stimulated glucose transport and PKC-ζ/λ phosphorylation/activation in vitro, but had no apparent effect on PI 3-kinase activation or PKB activation/phosphorylation in adipocytes isolated from GK rats. Rosiglitazone treatment in vivo also provoked increases in insulin-induced increases in phosphorylation and activation of PKC-ζ/λ and glucose transport in adipocytes isolated from nondiabetic, as well as GK-diabetic, rats, but, again, without altering PI 3-kinase activation or PKB activation/phosphorylation.

**EXPERIMENTAL PROCEDURES**

**Experimental Rats and Treatment with Rosiglitazone in Vivo—**A colony of GK rats, originally obtained from Drs. S. Suzuki and T. Toyoda (Tohoku University, Sendai, Japan), has been maintained in our vivarium for the past 7 years. All offspring in this colony have been consistently hyperglycemic, and peripheral insulin resistance in these offspring has been documented in clamp studies (21). As controls for GK-diabetic rats, we used nondiabetic Wistar rats, which were purchased from Harlan Industries. We also used 10–14-week-old male nondiabetic Harlan Sprague-Dawley rats. All rats were fed the same diet and kept for at least 2 weeks prior to experimental use in the same environment in our vivarium, which is temperature-controlled and diet and kept for at least 2 weeks prior to experimental use in the same environment in our vivarium, which is temperature-controlled and environment in our vivarium, which is temperature-controlled.

**Preparation and Incubations of Rat Adipocytes—**As described (12, 28), rat adipocytes were prepared by collagenase digestion of epididymal fat pads and incubated for indicated times in glucose-free Krebs-Ringer phosphate (KRP) medium containing 1% bovine serum albumin and indicated concentrations of insulin. After incubation, cells were used for studies of glucose transport (see below), or chilled and sonicated in the following buffers for assays of immunoprecipitable PKC-ζ/λ, PI 3-kinase, or PKB. For studies of PKC-ζ/λ activation, as described (12), 75 kDa PKC-luciferase was omitted from the PKC-ζ activation assay. For studies of PKB activation, buffer contained 250 mM sucrose, 20 mM Tris/HCl (pH 7.5), 1.2 mM MgCl₂, 200 μM b-mercaptoethanol, 1 mM phenylmethylsulphonyl fluoride (PMSF), 10 μg/ml aprotinin, 20 μg/ml leupeptin, 3 mM Na₃VO₄, 3 mM NaF, 3 mM Na₃P₂O₇, and 1 μM LR-microcystin. Homogenates were centrifuged for 10 min at 700 g to remove nuclei, cellular debris, and floating fat. Supernatants were then supplemented with (a) 0.15 M NaCl, 1% Triton X-100, and 0.5% Nonidet and used for immunoprecipitation of PKC-ζ/λ or PI 3-kinase, or with (b) 1% Triton X-100 for immunoprecipitation of PKB, as described below.

**Studies of Glucose Transport—**As described (10, 12, 28), adipocytes were equilibrated in glucose-free KRP medium and treated for 30 min with or without indicated concentrations of insulin, following which 2-deoxy-D-glucose (0.2 μM, 50 μCi; NEN Life Science Products) uptake was measured over a 1-min period.

**Studies of PKC-ζ/λ Activation and Autophosphorylation—**As described previously (12, 28), cell lysates were immunoprecipitated overnight at 0–4 °C with a polyclonal antiserum (Santa Cruz Biotechnologies; Santa Cruz, CA) that recognizes the C termini of both PKC-ζ and PKC-λ (as shown below, rat tissues primarily contain PKC-ζ, whereas mouse tissues primarily contain PKC-λ). Precipitates were collected on protein AG-Sepharose beads, washed, and incubated for 8 min at 30 °C in 10 μl of buffer containing 50 mM Tris/Cl (pH 7.5), 5 mM MgCl₂, 100 μM NaVO₃, 100 μM Na₃P₂O₇, 1 mM NaF, 100 μM PMSF, 50 μM ATP, 2.5 μCi of [γ-32P]ATP (NEN Life Science Products), 4 μg of actinomycin D, and 40 μg of the PKC-ζ pseudosubstrate (amino acids, 153–164) (Quality Controlled Biochemicals), as described previously (12, 28). In autophosphorylation assays, the exogenous substrate was omitted from the PKC-ζ/λ assay, and, after incubation, aliquots of the assay mixtures were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to Immobilon membranes, and assayed for PKC-ζ/λ by immunoblotting.

**RESULTS**

**Studies in Wistar Nondiabetic and GK-diabetic Rat Adipocytes**

**PKC-ζ/λ Activation and Phosphorylation—**Similar to findings in previous studies of adipocytes of Harlan Sprague-Dawley rats (12, 28), insulin provoked nearly a 2-fold increase (p < 0.001; see Fig. 1 legend for statistical methods) in immunoprecipitable PKC-ζ/λ enzyme activity in adipocytes isolated from Wistar nondiabetic rats (Fig. 1). Interestingly, the effect of insulin on PKC-ζ/λ activity was approximately 50% lower in adipocytes of GK-diabetic rats (p < 0.001, comparison to insulin effect on PKC-ζ/λ activity in adipocytes of Wistar nondiabetic rats), and, moreover, this defect in PKC-ζ/λ activation was more than completely reversed by 10–14 days of rosiglitazone treatment (p < 0.001). Of further note, rosiglitazone treatment provoked 20–30% increases (also see Harlan Sprague-Dawley studies below) in basal (not significant) and insulin-stimulated (p < 0.01) PKC-ζ/λ enzyme activity in adipocytes of Wistar nondiabetic, as well as GK-diabetic rats (Fig. 1). Alterations in PKC-ζ/λ enzyme activity were accompanied by similar alterations in the phosphorylation of threonine 410 in the activation loop of PKC-ζ. As seen in Fig. 2, in the absence of rosiglitazone treatment, insulin-stimulated threonine 410 phosphorylation was greater in adipocytes isolated from Wistar nondiabetic, as compared with GK-diabetic, rats; moreover, rosiglitazone treatment provoked increases in basal, as well as insulin-stimulated, threonine 410 phosphorylation in Wistar nondiabetic and, even more so, in GK-diabetic adipocytes.

**PI 3-Kinase Activation—**The decrease in insulin-induced ac-
tivation of PKC-ζ/λ in GK adipocytes was accompanied by decreased activation of IRS-1-dependent PI 3-kinase (Fig. 3). However, in contrast to PKC-ζ/λ, rosiglitazone did not alter basal or insulin-stimulated PI 3-kinase activity (Fig. 3).

PKB Activation—In association with defects in insulin-induced increases in PI 3-kinase activity and phosphorylation and activation of PKC-ζ/λ, insulin-induced activation of PKB, as assessed by measurement of PKB enzyme activity, appeared to be diminished mildly by approximately 20% (Fig. 4), although increases in immunoreactive phosphoserine 473-PKB (Fig. 5) did not appear to be altered in adipocytes isolated from GK diabetic rats. More importantly, rosiglitazone treatment was without effect on either PKB enzyme activity or phosphorylation in adipocytes isolated from either Wistar nondiabetic or GK diabetic rats (Figs. 4 and 5).

2-Deoxyglucose Uptake Studies—In conjunction with decreased activation of PKC-ζ/λ, maximal effects of insulin on 2-deoxyglucose uptake were diminished by approximately 50% in adipocytes isolated from GK diabetic rats, as compared with uptake observed in adipocytes isolated from Wistar nondiabetic rats; the $K_m$ value, however, was not altered (Fig. 6). Further, in association with the enhancement of insulin-induced PKC-ζ/λ activation in adipocytes obtained from both Wistar nondiabetic and GK diabetic rats, rosiglitazone treatment enhanced both the maximal and half-maximal effects of insulin on 2-deoxyglucose uptake in adipocytes of both Wistar nondiabetic and GK diabetic rats, and the presently observed defect in glucose transport in GK diabetic adipocytes was no longer apparent (Fig. 6).

The levels of immunoreactive PI 3-kinase/p85α subunit, PDK-1, PKB, pPKB (phosphoserine 473-PKB), combined PKC-ζ/λ, GLUT1, and GLUT4 were comparable, in adipocytes isolated from Wistar nondiabetic and GK diabetic rats, and were not altered significantly by rosiglitazone treatment (Fig. 5).

It may be noted that the recovery of (a) combined immunoreactive PKC-ζ/λ (assessed with an antiserum that recognizes the C terminus of both PKC-ζ and PKC-λ); (b) immunoreactive PKC-λ (assessed with antibodies that specifically recognize an internal sequence of PKC-λ); and (c) immunoreactive PKC-ζ (assessed with an antiserum that specifically recognizes a sequence in the N terminus of PKC-ζ) in PKC-ζ/λ immunoprecipitates was comparable in all experimental groups and was not altered by diabetes, rosiglitazone treatment, or insulin treatment (Fig. 5). Additionally, note that it was necessary to use relatively large amounts of lysates (1 mg) for immunoprecipitation to detect the seemingly strong signals for PKC-λ shown in Fig. 7. As also shown in Fig. 7, in comparing lesser amounts (75 μg) of whole cell lysates prepared from rat and mouse adipocytes, it is evident that, despite having comparable levels of total immunoreactive atypical PKC-ζ plus PKC-λ (PKC-ζ/λ blot), rat adipocytes are relatively rich in PKC-ζ, and mouse adipocytes are relatively rich in PKC-λ.
cytes of Harlan Sprague-Dawley nondiabetic rats (Fig. 9).

Rats were treated with Wistar nondiabetic and GK-diabetic rats.

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, presumably involving threonine 560 (Fig. 9).

FIG.3.

FIG.4.

Effects of insulin and rosiglitazone (RSGZ) on PKB enzyme activity in adipocytes isolated from Wistar nondiabetic and GK-diabetic rats. Experiments were conducted as in Figs. 1–4, except that, after incubation, immunoprecipitable PKB enzyme activity was measured as described under “Experimental Procedures.” Shown here are mean ± S.E. of three determinations.

Studies in Harlan Sprague-Dawley Nondiabetic Adipocytes

The effects of rosiglitazone in nondiabetic adipocytes were explored further in Harlan Sprague-Dawley rats. As seen in Fig. 8, rosiglitazone treatment for 6–7 days provoked 45% and 65% increases in basal and insulin-stimulated PKC-ζ/λ enzyme activity, and the increment in PKC-ζ/λ enzyme activity owing to insulin treatment was increased by 87% (PKC-ζ/λ activity in each of the four groups in Fig. 8 was significantly different, i.e. p < 0.05, from all other groups, as determined by one-way analysis of variance and the Sheffe post hoc test for multiple comparisons. (Note: the reason for greater effects of rosiglitazone in adipocytes of Harlan Sprague-Dawley rats, as compared with those of Wistar rats, is presently uncertain, but could be due to strain differences, shorter duration of rosiglitazone treatment, and/or the greater sample size in the Harlan Sprague-Dawley study.). In contrast to the enhancement of insulin-induced activation of PKC-ζ/λ, the activation of phosphotyrosine-associated PI 3-kinase (Fig. 9) and the phosphorylation of serine 473 in PKB (Figs. 8 and 9) were not enhanced by 6–7-day rosiglitazone treatment.

In association with rosiglitazone-induced increases in PKC-ζ/λ enzyme activity in adipocytes isolated from Harlan Sprague-Dawley nondiabetic rats, rosiglitazone treatment provoked increases in basal and insulin-stimulated levels of (a) PDK-1-dependent phosphorylation of threonine 410 in the activation loop of PKC-ζ (Figs. 8 and 9) and (b) subsequent autophosphorylation of PKC-ζ, presumably involving threonine 560 (Fig. 9). Additionally, as in adipocytes of Wistar nondiabetic rats, rosiglitazone treatment provoked increases in insulin-stimulated glucose transport in adipocytes isolated from Harlan Sprague-Dawley nondiabetic rats (Fig. 6).

As in Wistar-nondiabetic adipocytes, rosiglitazone treatment had no effects on the levels of immunoreactive PI 3-kinase/p85α subunit, PKD-1, PKB, PKC-ζ/λ, GLUT1, and GLUT4 in adipocytes of Harlan Sprague-Dawley nondiabetic rats (Fig. 9).

DISCUSSION

It was of considerable interest to find that the activation of PKC-ζ/λ by insulin was impaired in adipocytes of GK-diabetic rats. This defect could not be explained by reduced levels of PKC-ζ, PKC-λ, PDK-1, or the p85α subunit of PI 3-kinase. On the other hand, this defect in insulin-stimulated PKC-ζ/λ enzyme activity in GK adipocytes was associated with decreases in IRS-1-dependent PI 3-kinase activation and PDK-1-dependent phosphorylation of threonine 410 in the activation loop of PKC-ζ. It is therefore reasonable to suggest that the GK-diabetic state led to a diminution in the activity or action of factors that are upstream of PKC-ζ, i.e. PI 3-kinase and PDK-1, which, in turn, diminished PKC-ζ/λ phosphorylation and activ-
ity; however, in this scenario, since insulin-stimulated activation of PKB was inhibited to a lesser degree than PKC-ζ/λ, it would be necessary to postulate that, as compared with PKC-ζ/λ, PKB is more effectively activated at lower levels of PI 3-kinase activation, or there are separate pools of upstream signaling factors, including PI 3-kinase and PDK-1, that regulate PKC-ζ/λ and PKB. As another alternative, it is possible that the diabetic state, as existing in GK rats, may be attended by an increase in the activity of a factor that negatively modulates PKC-ζ/λ, e.g. by dephosphorylation of the activation loop and/or autophosphorylation sites, or by inhibition of the catalytic or substrate-binding sites of both PKC-ζ/λ, irrespective of, or in addition to, PI 3-kinase and PDK-1 activation. In this regard, it is of interest that (a) okadaic acid, which inhibits protein phosphatase-2A (PP2A), activates PKC-ζ/λ (29), and (b) increases in basal cytosolic PP2A activity, as well as diminished inhibition of PP2A activity in response to acute treatment with insulin, have been observed in adipocytes of GK-diabetic rats (23). Further studies are needed to more fully define the mechanism of inhibition of insulin-stimulated PKC-ζ/λ activation in adipocytes of GK-diabetic rats.

It was also of considerable interest that rosiglitazone not only corrected the defects in insulin-stimulated PKC-ζ threonine 410 phosphorylation and enzymatic activity of PKC-ζ/λ in adipocytes of GK-diabetic rats, but also increased the phosphorylation of the threonine 410 loop and threonine 560 autophosphorylation sites of PKC-ζ/λ, as well as the enzymatic activity of PKC-ζ/λ, in adipocytes of nondiabetic rats. Moreover, these increases in PKC-ζ/λ phosphorylation and activation were not associated with alterations in IRS-1-dependent PI 3-kinase activity or activation by insulin in diabetic and nondiabetic adipocytes. These findings suggested that (a) rosiglitazone provoked an alteration in a factor that either enhanced the phosphorylation, or diminished the dephosphorylation, of loop and autophosphorylation sites in PKC-ζ/λ, and (b) stimulatory effects of rosiglitazone on PKC-ζ/λ were not explicable simply on the basis of reversing the action of a uniquely diabetes-associated inhibitory factor.

The mechanism whereby rosiglitazone enhanced phosphorylation and subsequent activation of PKC-ζ/λ is uncertain. Since phosphorylation of threonine 410 in the activation loop of PKC-ζ was enhanced by rosiglitazone, as alluded to above, it is possible that the activity or action of PDK-1, which, along with PIP3, regulates this loop phosphorylation (12, 28, 30, 31), may have been increased by rosiglitazone treatment. In evaluating this possibility, it may be noted that increases in threonine 410 phosphorylation of threonine 410 in the activation loop of PKC-ζ.
Thiazolidinediones Activate Atypical PKCs

FIG. 8. Effects of insulin and rosiglitazone (RSGZ) treatment on PKC-ζ/λ enzyme activity, phosphorylation of threonine 410 in PKC-ζ, and phosphorylation of serine 473 in PKB in adipocytes prepared from nondiabetic Harlan Sprague-Dawley rats. As described under “Experimental Procedures,” rats were treated in vivo with rosiglitazone for 6–7 days, and adipocytes were isolated from treated and untreated rats and incubated in glucose-free KRP medium for 10 min with or without 10 nm insulin, after which cell lysates were examined for changes in PKC-ζ/λ enzyme activity (panel A), and, by Western analysis, for threonine 410 phosphorylation (panel B) and PKB serine 473 phosphorylation (panel C). Bar graphs and brackets reflect mean ± S.E. of (n) determinations.

FIG. 9. Effects of insulin and rosiglitazone (RSGZ) treatment on PI 3-kinase activity, phosphorylation of threonine 410 in PKC-ζ, and autophosphorylation of PKC-ζ (right) and levels of immunoreactive PI 3-kinase p85α subunit (P13K), PDK-1 (PDK), PKB, phosphoserine 437-PKB (pPKB), PKC-ζ/λ, GLUT1, and GLUT4 (left) in adipocytes prepared from nondiabetic Harlan Sprague-Dawley rats. As described under “Experimental Procedures,” rats were treated in vivo with rosiglitazone for 6–7 days, and adipocytes were isolated from treated and untreated rats and incubated in glucose-free KRP medium for 10 min with or without 10 nm insulin, after which cell lysates were examined for changes in immunoreactive PI 3-kinase p85α subunit, PDK-1, PKB, PPK, PKC-ζ/λ, and GLUT1 and GLUT4 glucose transporters, as shown in the left panel. Shown on the right, (a) the P13P autoradiogram reflects radioactivity recovered in PI-3-PO4 following assay of phosphotyrosine-associated PI 3-kinase, lipid extraction, and thin layer chromatography; (b) the α-p410 immunoblot reflects immunoreactive phosphothreonine 410 in PKC-ζ following resolution on SDS-PAGE; and (c) the 32P-P/ζ autoradiogram reflects autophosphorylation of 32P-labeled PKC-ζ following assay and resolution on SDS-PAGE. Autoradiograms and immunoblots are representative of at least four determinations. See also Fig. 8 for mean values and standard errors for PKC-ζ enzyme activity, threonine 410 phosphorylation in PKC-ζ, and phosphoserine 473 phosphorylation in PKB as observed in multiple samples.

loop phosphorylation in the basal and insulin-stimulated state were not always paralleled by strictly proportional increases in PKC-ζ/λ enzyme activity, or, for that matter, in glucose transport. This lack of strict proportionality may reflect the fact that, in addition to increases in PDK-1 activity, the full activation of PKC-ζ/λ requires (a) PIP3- dependent enhancement of autophosphorylation of threonine 560 (28), as presently observed with rosiglitazone treatment, and (b) PIP3-dependent conformational changes that are phosphorylation-independent, most likely involving the relief of pseudosubstrate autoinhibition; moreover, increases in specific insulin-sensitive pools of membrane-associated PIP3 and PIP3-dependent kinases, such as PKC-ζ/λ and PKB, are probably of major importance in the activation of glucose transport. Additionally, in evaluating the possibility that rosiglitazone may have acted via PDK-1, it is important to note that we did not detect significant effects of rosiglitazone on PI 3-kinase activation, or on phosphorylation or activation of PKB, which, like PKC-ζ/λ, is regulated by PDK-1 (32, 33). It therefore seems unlikely that rosiglitazone provoked a generalized increase in PDK-1 activity or action. On the other hand, it is possible that rosiglitazone may have (a) acted on a pool of PDK-1 that specifically regulates PKC-ζ/λ, or (b) up-regulated or down-regulated a factor(s) that selectively modulates PKC-ζ/λ loop phosphorylation, in conjunction with, but independently of, PDK-1. Further studies are needed to (a) identify the rosiglitazone-sensitive factor(s) that regulates or modulates PKC-ζ/λ phosphorylation and activation basally and in response to insulin stimulation, and (b) examine the relationship of the rosiglitazone-sensitive stimulatory factor(s) to the diabetes-dependent inhibitory factor(s).

It should be noted that the marked improvement in insulin- induced PKC-ζ/λ enzyme activation in adipocytes of GK-diabetic rats observed following rosiglitazone treatment occurred despite only modest decreases in serum glucose levels, namely from approximately 329 ± 9 (n = 15) to 284 ± 9 (n = 8) mg/dl (see also Ref. 22). It therefore seems unlikely that rosiglitazone effects on PKC-ζ/λ in adipocytes of GK rats can be explained simply by improvement of glucotoxicity. It should also be noted that there were no significant changes in serum glucose levels in nondiabetic rats following rosiglitazone treatment for either 6 or 14 days. These findings of independence of presently observed changes in PKC-ζ/λ activation on serum glucose levels, however, does not negate the possibility that such improvement in PKC-ζ/λ activation might also occur through improvement in glucotoxicity.

In contrast to apparent correlations with PKC-ζ/λ activation, rosiglitazone-induced increases in insulin-stimulated glucose transport in adipocytes of both nondiabetic and GK-diabetic rats could not be correlated to gross alterations in the levels of either GLUT1 or GLUT4 glucose transporters. (b) changes in IRS-1-dependent PI 3-kinase activation, or (c) changes in the phosphorylation/activation of PKB. On the other hand, there may be specific pools of these factors that are not reflected in the gross overall assays. In any event, the present findings are compatible with the possibility that increases in insulin-stimulated glucose transport following rosiglitazone treatment in adipocytes of nondiabetic and GK-diabetic rats may have been at least partly due to increases in the phosphorylation and

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enzymatic activation of PKC-ζ. Obviously, further studies are needed to test this hypothesis.

In summary, insulin-stimulated glucose transport, IRS-1-dependent PI 3-kinase activation, and phosphorylation/activation of PKC-ζ were found to be defective in adipocytes of type II diabetic GK rats. Of particular interest, these defects in insulin-stimulated glucose transport and PKC-ζ phosphorylation/activation were fully or more than fully reversed by rosiglitazone treatment, despite the fact that rosiglitazone had little or no significant effect on the activation of IRS-1-dependent PI 3-kinase or PKB. Similarly, in adipocytes of non diabetic rats, rosiglitazone increased (a) insulin-stimulated glucose transport, (b) insulin effects on the phosphorylation of threonine 410 loop and threonine 560 autophosphorylation sites in PKC-ζ, and enzymatic activation of PKC-ζ, but was without effect on PI 3-kinase or PKB activation. Our findings are compatible with the possibility that increases in insulin-induced PKC-ζ phosphorylation and activation contributed importantly to increases in glucose transport observed in isolated adipocytes following rosiglitazone treatment of GK-diabetic and nondiabetic rats. Further studies are needed (a) to see if rosiglitazone and other TZDs alter PKC-ζ activation in other animal and human states of type II diabetes mellitus, obesity, and other forms of clinical insulin resistance, and (b) to more fully define the mechanism that is responsible for increased phosphorylation and activation of PKC-ζ following TZD treatment.

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