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Regulation of Insulin-Stimulated Tyrosine Phosphorylation of Shc and Shc/Grb2 Association in Liver, Muscle, and Adipose Tissue of Epinephrine- and Streptozotocin-Treated Rats

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Shc protein phosphorylation has been extensively characterized as the initial step that activates a complex mitogenic pathway through its association with Grb2. In the present study, we investigated the adrenergic control of insulin-induced Shc phosphorylation and Shc-Grb2 association, and the modulating effect of streptozotocin-induced diabetes mellitus on Shc phosphorylation and Shc/Grb2 association. Acute treatment with epinephrine, which leads to a normoglycemic insulin-resistant state, does not affect insulin-induced Shc tyrosine phosphorylation or Shc-Grb2 association in liver, muscle, or fat. By contrast, a significant increase in insulin-induced Shc phosphorylation is observed in liver and muscle of rats treated with streptozotocin. The association of Shc/Grb2 is also increased in both tissues following insulin treatment. These data suggest that while epinephrine preserves the insulin-induced phosphorylation of Shc and the mitogenic pathway stimulated by Shc-Grb2 association, treatment with streptozotocin leads to a tissue-specific increase in the activity of the initial step that ultimately results in the activation of the Shc/Grb2 mitogenic pathway.

Key Words: Epinephrine; diabetes mellitus; streptozotocin; insulin receptor substrate-1; Shc; Shc-Grb2 association.

Introduction

The insulin receptor (IR) is a protein with endogenous tyrosine kinase activity that following the activation by insulin undergoes rapid autophosphorylation and phosphorylates intracellular protein substrates, including insulin receptor substrates (IRSs) 1–4 and Shc *(1–5)*. Tyrosine-phosphorylated IR couples to downstream SH2- or SH3-containing signaling molecules, through the phosphotyrosyl residues of IRSs. The p85 regulatory subunit of PI 3-kinase *(6)*, Grb2 *(7)*, SHP2 (Syp) *(8)*, and Nck *(9)* are some of the proteins regulated by this pathway. Like IRSs, Shc protein is also an IR substrate *(5,10)* that acts as a docking molecule for the SH2/SH3 adapter protein Grb2 *(11,12)*. Shc/Grb2 association is upstream to the activation of an important signaling pathway leading to the stimulation of mitogen-activated protein kinase (MAPK) *(13–15)*. We have reported recently that insulin can induce time- and dose-dependent Shc tyrosine phosphorylation and association with Grb2 in rat tissues *(16)*, and that in some situations of insulin resistance there is an increased correlation between insulin levels and Shc tyrosine phosphorylation *(17)*. Thus, IRSs and Shc constitute downstream coupling molecules that connect the activated receptor to distal events.

Insulin resistance is defined as a subnormal biologic response to a given concentration of insulin. Insulin resistance is characteristic of many disease states such as type 2 diabetes, uncontrolled type 1 diabetes, obesity, and hypertension. Supernormal concentrations of epinephrine are also known to cause insulin resistance *(18–20)*. Previous studies have demonstrated that in streptozotocin-induced diabetes (an animal model of type 1 diabetes), there is increased IRS-1 tyrosine phosphorylation and association with PI 3-kinase *(18,21)*. By contrast, in rats treated with an excess of epinephrine, there is a reduction in the insulin-induced IRS-1 phosphorylation level and in the association with PI 3-kinase *(20)*. These data demonstrate that IRS-1 is specifically regulated in animal models of insulin resistance and may play a critical role in the altered insulin action observed in such models. In addition, fatty acid–induced insulin resistance was recently associated with a decrease in insulin-stimulated MAPK *(22)*. Tumor necrosis factor-α (TNF-α) and hyperglycemia also regulate Shc phosphorylation in cultured cells *(23)*, suggesting that Shc may be involved in the molecular mechanisms of insulin resistance. However, the regulation of Shc in tissues from streptozotocin- and epinephrine-treated rats has not yet been investigated.

In the present study, we examined the insulin-induced Shc tyrosine phosphorylation and association with Grb2 in liver, muscle, and fat of streptozotocin- and epinephrine-treated rats.

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Table 1
Characteristics of Rats Studied[a]

Groups	*n*	Body weight (g)	Basal plasma glucose (mg/dL)	Basal serum insulin μU/mL)
Control	20	148 ± 3	115 ± 2	29 ± 2
Epinephrine	12	144 ± 3	117 ± 3	41 ± 4
Streptozotocin diabetes	16	102 ± 3*	452 ± 2*	6 ± 6*

[a] Data are expressed as the mean ± SEM.
*$p \leq 0.05$ compared to the corresponding control.

Results

Characteristics of Epinephrine- and Streptozotocin-Treated Rats

To establish the degree of insulin resistance in epinephrine-treated rats, 10 rats from each experimental group and their respective normal matching (nontreated rats) underwent an insulin tolerance test. The plasma glucose disappearance rate (k_{itt}) of the nontreated group was 4.40 ± 0.39%/min. Acute treatment with epinephrine induced insulin resistance, as reflected in the glucose disappearance rate of 1.89 ± 0.63%/min ($p < 0.05$ related to the nontreate group). In streptozotocin-treated rats, plasma glucose and serum insulin levels were measured, and rats were considered diabetic when glucose was >300 mg/dL. The glucose disappearance rate of streptozotocin-treated diabetic rats was 1.94 ± 0.81%/min ($p < 0.01$ related to nontreated group). Table 1 summarizes the body weight and plasma glucose and serum insulin levels of control, epinephrine-, and streptozotocin-treated rats.

Shc Protein Concentration in Tissues of Diabetic or Epinephrine-Treated Rats

Shc protein concentration in liver, muscle, and adipose tissue of streptozotocin-treated diabetic rats and epinephrine-treated rats was not significantly different from the respective controls before and after acute stimulation with insulin (data not shown). The predominant isoform observed in muscle was $p52^{Shc}$, whereas in liver and fat $p52^{Shc}$ was the only phosphorylated isoform detected. For all the experiments described, the controls are defined as the normal-matching rats subjected to insulin injection, and basal means normal or treated animals not subjected to insulin injection.

Effect of Epinephrine on Shc Phosphorylation and Shc/Grb2 Association

Insulin-induced tyrosine phosphorylation of Shc in liver of epinephrine-treated rats did not differ from that of control rats (100 ± 9% for controls vs 85 ± 10% for epinephrine treated; $p = 0.2$) (Fig. 1A, left). In this tissue, the levels of insulin-induced Shc/Grb2 association of epinephrine-treated rats did not show any difference when compared with their controls (100 ± 23% for controls vs 74 ± 2% for epinephrine treated; $p = 0.9$) (Fig. 1A, right). Basal levels of Shc/Grb2 association in liver of epinephrine-treated rats increased significantly (19 ± 5% for normal vs 69 ± 6% for epinephrine treated; 69% ± 6%; $p < 0.003$), when compared with basal levels of nonepinephrine-treated rats (Fig. 1A, right).

Insulin-induced tyrosyl phosphorylation of Shc in the muscle of epinephrine-treated rats was similar to that of the controls (100 ± 8% for controls vs 103 ± 8% for epinephrine treated; $p = 0.4$) (Fig. 1B, left). Insulin-induced Shc/Grb2 association levels in muscle of epinephrine-treated rats did not differ from those for the controls (100 ± 5% for controls vs 97 ± 1% for epinephrine treated; $p = 0.21$) (Fig. 1B, right). A significant increase was observed in the basal levels of Shc/Grb2 association in muscle of epinephrine-treated rats in comparison with basal levels of normal animals (23 ± 5% for normal vs 87 ± 9% for epinephrine treated; $p < 0.05$).

In the adipose tissue of epinephrine-treated rats, the levels of insulin-induced Shc protein phosphorylation were similar to the levels observed in their controls (100 ± 6% for controls vs 91 ± 15% for epinephrine treated; $p = 0.8$) (Fig. 1C, left). In these animals, the insulin-induced Shc/Grb2 association in adipose tissue was similar to that of controls (100 ± 6%, for controls vs 96 ± 4% for epinephrine treated; $p = 0.2$) (Fig. 1C, right). Basal levels of Shc/Grb2 association in fat of epinephrine-treated rats were significantly increased (37 ± 17% for normal vs 86 ± 4% for epinephrine treated; $p = 0.05$) (Fig. 1C, right).

Effect of Streptozotocin on Shc Phosphorylation and Shc/Grb2 Association

Insulin-induced tyrosine phosphorylation levels of Shc in liver of streptozotocin-treated rats were significantly higher compared with controls (100 ± 7% for controls vs 175 ± 5% for streptozotocin treated; $p < 0.001$) (Fig. 2A, left). The extent of insulin-induced Shc/Grb2 association was also increased (100 ± 13% for controls vs 194 ± 8% for streptozotocin treated; $p < 0.001$) (Fig. 2A, right).

In muscle of streptozotocin-treated rats, there was an increase in the basal phosphorylation of Shc compared with nontreated rats (21 ± 3% for normal vs 87 ± 8% for streptozotocin treated; $p < 0.05$) (Fig. 2B, left), which was not accompanied by an increase in Shc/Grb2 association before the injection of insulin. Insulin-induced Shc tyrosine phosphorylation was higher in muscle of streptozotocin-treated diabetic rats (100 ± 11% for controls vs 180 ± 12% for streptozotocin treated; $p < 0.01$) (Fig. 2B, left), with a corresponding increase in the association between Shc and Grb2 (100 ± 18% for controls vs 162 ± 8% for streptozotocin treated; $p < 0.01$) (Fig 2B, right).

In adipose tissue from streptozotocin-treated rats, there was a nonsignificant increase in insulin-induced Shc phosphorylation levels compared with controls (100 ± 4% for

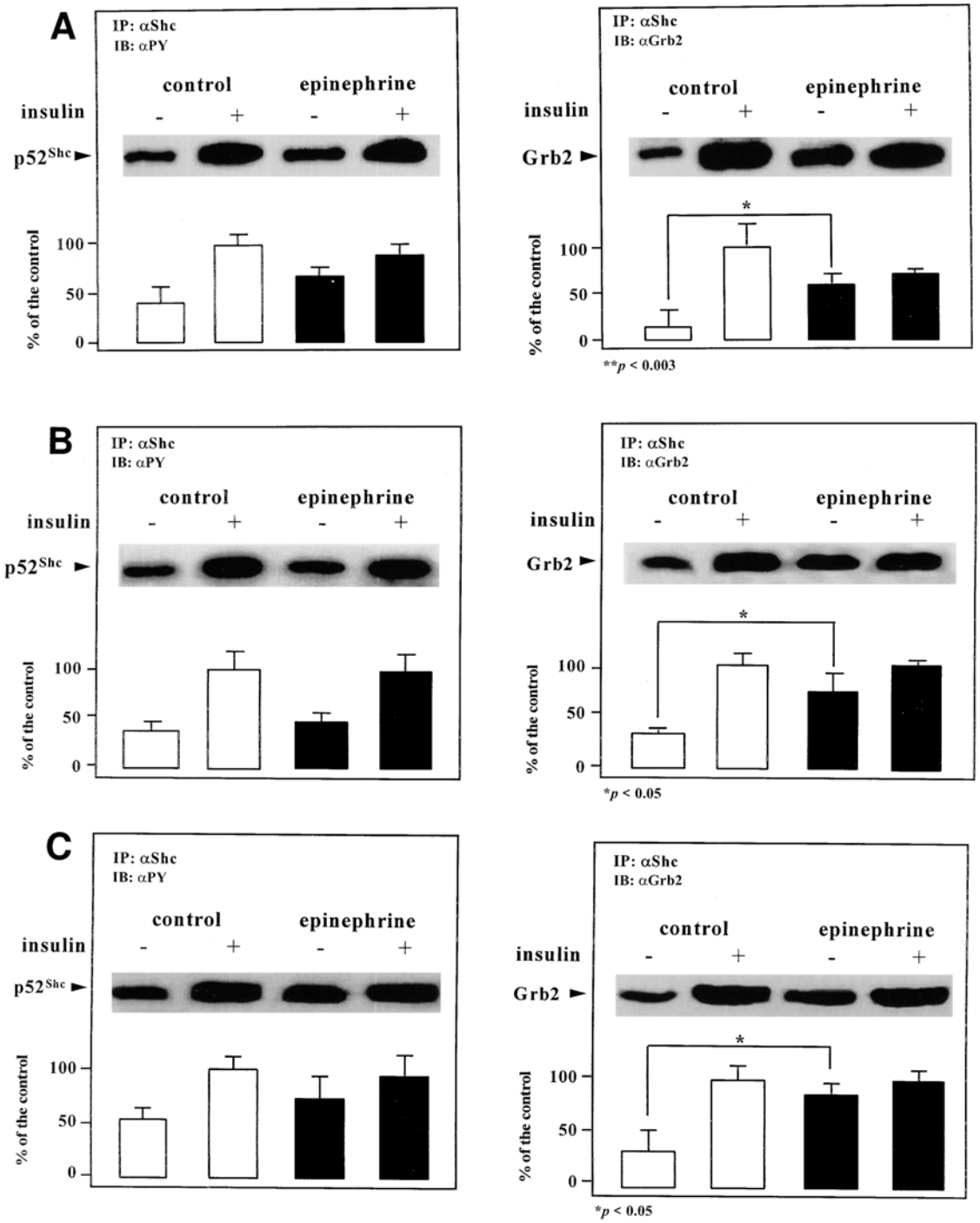

Fig 1. Insulin stimulates tyrosine phosphorylation of Shc and Shc/Grb2 association in tissues from epinephrine-treated rats. Rats were anesthetized and the abdominal wall was incised to expose the viscera. Saline or 60 μg of insulin was administered as a bolus injection into the portal vein. Five minutes later, a sample of the tissue was excised and homogenized in extraction buffer at 4ºC as described in Materials and Methods. After centrifugation, aliquots containing equal amounts of protein were immunoprecipitated (IP) with anti-Shc antibodies. The immunoprecipitates were separated on 10% SDS-polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were then immunoblotted (IB) sequentially with antiphosphotyrosine antibody (**left**) or anti-Grb2 antibody (**right**). Proteins were isolated and processed as described in Materials and Methods. (**A**) Liver; (**B**) muscle; (**C**) adipose tissue extracts. The levels of Shc tyrosyl phosphorylation and Shc-Grb2 association were determined by scanning densitometry of six separate experiments for each tissue and were expressed as a percentage of the insulin-stimulated control (100%). Columns and bars represent the mean ± SEM ($p < 0.05$).

controls vs 133 ± 9% for streptozotocin treated; $p = 0.2$) (Fig. 2C, left). The insulin-induced Shc/Grb2 association was consistent with the Shc tyrosyl phosphorylation levels and did not differ from the controls (100 ± 6% for controls vs 125 ± 8% for streptozotocin treated; $p = 0.1$) (Fig. 2C, right).

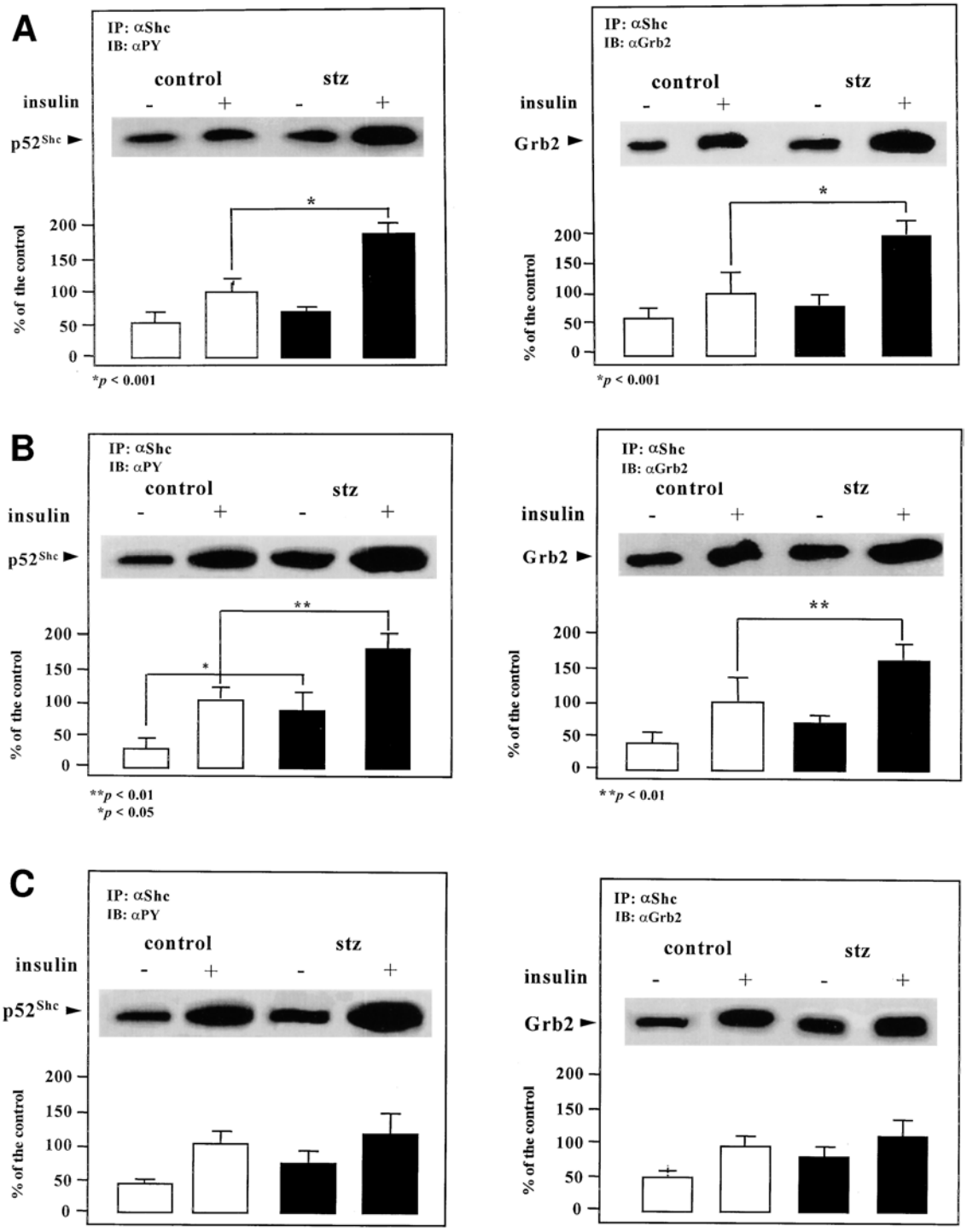

Fig 2. Insulin stimulates tyrosine phosphorylation of Shc and Shc/Grb2 association in tissues from streptozotocin (stz)-treated rats. Rats were anesthetized and the abdominal wall was incised to expose the viscera. Saline or 60 μg of insulin was administered as a bolus injection into the portal vein. Five minutes later, a sample of the tissue was excised and homogenized in extraction buffer at 4ºC as described in Materials and Methods. After centrifugation, aliquots containing equal amounts of protein were immunoprecipitated (IP) with anti-Shc antibodies. The immunoprecipitates were separated on 10% SDS-polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were then immunoblotted (IB) sequentially with antiphosphotyrosine antibody (**left**) or anti-Grb2 antibody (**right**). Proteins were isolated and processed as described in Materials and Methods. (**A**) Liver; (**B**) muscle; (**C**) adipose tissue extracts. The levels of Shc tyrosyl phosphorylation and Shc-Grb2 association were determined by scanning densitometry of six separate experiments for each tissue and were expressed as a percentage of the insulin-stimulated control (100%). Columns and bars represent the mean ± SEM ($p < 0.05$).

There was some degree of variability in band intensity among different experiments, which might be owing to radioisotope half-life, RX exposition, and biologic variability of experimental animals. This was corrected by performing the analysis as a variation of insulin-stimulated control.

Discussion

Following insulin stimulation, several proteins undergo tyrosine phosphorylation, including the β-chain of the IR, IRS-1, IRS-2, and Shc *(24)*. Shc belongs to a family of signal-transduction proteins implicated in several intracellular interactions between different signaling systems *(15)*. Once phosphorylated, Shc binds to the adapter protein Grb2, acting as a complex that docks different proteins to enhance or counterregulate the tyrosine kinase receptor's actions over specific mitogenic processes *(11–15)*.

In the present study, we analyzed the insulin-induced phosphorylation of $p52^{Shc}$ and the relationship between insulin-induced Shc phosphorylation and Shc/Grb2 association in liver, muscle, and fat of rats treated acutely with epinephrine or with streptozotocin. Insulin resistance in both groups was demonstrated by a decrease in the glucose disappearance rate after insulin infusion *(25)*. The insulin-induced tyrosine phosphorylation levels of Shc, and the Shc/Grb2 association, were similar in liver, muscle, and fat of acute epinephrine-treated rats compared with controls. However, there was a significant increase in basal Shc/Grb2 association levels in the three tissues studied following treatment with epinephrine. Thus, although the total amounts of Shc/Grb association in response to insulin in both epinephrine-treated and control rats were not different, the change in Shc/Grb association obtained in response to insulin relative to the corresponding basal state was reduced in the epinephrine-treated groups.

Comparison of these results with previous studies done under similar conditions *(20,26,27)*, revealed a clear dissociation between insulin-induced Shc and IRS-1 tyrosine phosphorylation in epinephrine-treated rats in the tissues examined. Indeed, whereas the insulin-induced tyrosine phosphorylation levels of IRS-1 in liver and muscle of epinephrine-treated rats were lower compared with controls *(20)*, the insulin-induced Shc phosphorylation in the same tissues was similar to that of controls. The decrease in IRS-1 tyrosine phosphorylation in epinephrine-treated rats correlated with that of the IR *(20)*. The mechanism by which a reduction in IR phosphorylation differentially regulates its own substrates is unknown. Shc can directly associate with the IR by binding to phosphorylated Tyr-960 in the receptor juxtamembrane region *(10)*, which is the same binding site for IRS-1 *(28)*. IRS-1 may compete for this tyrosine residue during association with the phosphorylated receptor *(29)*. It is possible that the serine phosphorylation of IRS-1 induced by an increase in intracellular cyclic adenosine monophosphate levels following treatment with epinephrine may reduce its binding affinity for the insulin receptor, allowing Shc to be more competitive. In support of this hypothesis, Li and Goldstein *(30)* have shown that reducing IRS-1 serine phosphorylation results in increased insulin-induced IRS-1 tyrosine phosphorylation and decreased Shc tyrosine phosphorylation.

The dissociation of insulin-induced IRS-1 and Shc tyrosine phosphorylation observed after acute treatment with epinephrine has been described in cell lines with mutations at two tyrosine phosphorylation sites on the IR *(31)*. Such cell lines showed insulin-induced tyrosine phosphorylation of Shc, Shc/Grb2 complex formation and p21ras-GTP formation, but had reduced tyrosine phosphorylation of IRS-1 and reduced IRS-1 association with Grb2 and PI 3-kinase. These results, together with our own, suggest that a reduction in IR phosphorylation may differentially induce postreceptor processes by preserving the phosphorylation of some, but not all, substrates and pathways.

The increased Shc-Grb2 association during the basal state in liver, muscle, and adipose tissue of epinephrine-treated rats suggests that epinephrine-induced tyrosine phosphorylation of p52 Shc leads to its association with Grb2 and MAPK activation. Studies showing that norepinephrine, through the α-adrenergic receptor, directly stimulates p52Shc and MAPK in human vascular smooth muscle cells support this hypothesis *(32)*. High levels of TNF-α owing to treatment with epinephrine may also contribute to the increase in basal Shc-Grb2 association *(23)*.

We also studied the effect of streptozotocin-induced diabetes mellitus, which is an insulin-resistant state characterized by deficient insulin production and action, on the regulation of Shc. In this state, high glucose levels were detected, and Shc phosphorylation was increased, so that the Shc/Grb2 association after stimulation with insulin was considerably augmented in liver and muscle and, to a lesser extent, in rat adipose tissue. In streptozotocin-treated diabetic animals under similar conditions, there was also an increase in insulin-induced IRS-1 tyrosine phosphorylation, as well as IRS-1-associated PI-3 kinase activity.

The mechanism responsible for the increase in IRS-1 and Shc tyrosine phosphorylation in liver and muscle of streptozotocin-treated diabetic animals is not known, but may it be related to the increase in IR number and phosphorylation *(18)*. However, in another animal model of insulin resistance with hypoinsulinemia (72-h fasted rats), in which there is an increase in IR number and phosphorylation, the increase in IRS-1 tyrosine phosphorylation is not accompanied by an increase in Shc phosphorylation. Since the difference between these two models is related mainly to glucose levels, it is possible that high glucose levels, perhaps resulting from an increase in the osmolarity, may contribute to the increased Shc phosphorylation level and association with Grb2 in liver and muscle *(33)*. The upregulation of substrate tyrosine phosphorylation in the presence of hypoinsulinemia could also potentially result from the reduced

activity of a phosphotyrosine-protein phosphatase, which is known to be decreased in the skeletal muscle of streptozotocin-treated diabetic rats *(34–36)*.

The increase in IRS-1 and Shc tyrosine phosphorylation in streptozotocin-treated diabetic animals occurs despite the state of decreased insulin responsiveness in both liver and peripheral tissues of these animals *(37–39)*. The fact that the glucose disappearance rate was lower in streptozotocin-treated diabetic rats suggests that tissue or circulating factors such as fatty acids, ketones, counterregulatory hormones, and acidosis may antagonize the stimulatory action of insulin on glucose uptake and metabolism, as previously discussed. It is possible that the increase in IRS-1 and Shc phosphorylation in liver and muscle of streptozotocin-induced diabetes could be a response to the impairment of insulin action.

The results of our study suggest that while epinephrine preserves the insulin-induced phosphorylation of Shc and the mitogenic pathway stimulated by Shc/Grb2 association, treatment with streptozotocin leads to a tissue-specific increase in the activity of the initial step that ultimately results in the activation of the Shc/Grb2 mitogenic pathway.

Materials and Methods

Materials

The reagents and apparatus for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were obtained from Bio-Rad (Richmond, CA). Tris, phenylmethylsulfonyl fluoride (PMSF), aprotinin, silicone, dithiothreitol (DTT), and streptozotocin (*N*-[methylnitrosocarbamoyl]-D-glucosamine) were obtained from Sigma (St. Louis, MO). Sodium amobarbital was purchased from Eli Lilly (Indianapolis, IN), and regular highly purified insulin (Iolin R) was from Biobrás (MG., Brazil). Protein A-Sepharose 6 MB was purchased from Pharmacia (Uppsala, Sweden). [^{125}I]-Protein A was from Amersham (Aylesbury, UK), and nitrocellulose (BA85; 0.2 µm) was obtained from Schleicher & Schuell (Keene, NH). Male Wistar rats were from the university's Animal Breeding Center. Monoclonal antiphosphotyrosine antibodies (1 µg/mL) were obtained from Upstate Biotechnology (Lake Placid, NY). Anti-Shc and anti-Grb2 rabbit polyclonal antibodies (100 µg/mL) were from Santa Cruz Biotechnology (Santa Cruz, CA).

Animals

Six-week-old Wistar rats (mean body weight of 148 ± 3 g) were fed standard rodent chow and water ad libitum. Food was withdrawn 12–14 h before the experiments. Diabetes was induced with streptozotocin dissolved in citrate buffer (pH 4.5) and administered intravenously in a single dose of 60 mg/kg of body weight to rats fasted overnight. Glucose levels were measured from the second day after treatment, by collecting a blood sample from tail vein. Experiments were performed 7 d after treatment with streptozotocin, as long as diabetes was demonstrated by plasmatic glucose increase and insulin decrease. Acute treatment with epinephrine involved a single injection of 25 µg of 5% (w/v) epinephrine/100 g of body weight given 10 min before the experimental procedure. All experiments with animals were approved by the Ethics Committee of the State University of Campinas (UNICAMP).

Methods

The rats were anesthetized with sodium thiopental intraperitoneally (100 µg/kg of body weight) and used 10–15 min later, as soon as anesthesia was indicated by the loss of foot and corneal reflexes. The abdominal cavity was opened, the portal vein was exposed, and 6 µg of insulin was injected. Samples of liver, skeletal muscle, and adipose tissue were collected 5 min after the insulin infusion, minced coarsely, and homogenized immediately in approx 10 vol of solubilization buffer A at 4ºC, using a Polytron PTA 20S homogenizer (model PT 10/35; Brinkmann), operated at maximum speed for 30 s. Buffer A (for solubilization) consisted of 1% Triton X-100, 50 m*M* HEPES (pH 7.4), 100 m*M* sodium pyrophosphate, 100 m*M* sodium fluoride, 10 m*M* EDTA, 10 m*M* sodium vanadate, 2 m*M* PMSF, and 0.1 mg of aprotinin/mL.

For both experimental models studied, the tissues were extracted in an identical manner. The extracts were centrifuged at 30,000*g* in a Beckman 70.1 Ti rotor at 4ºC for 20 min to remove insoluble material, and the resulting supernatant was used for immunoprecipitation with 10 µL of polyclonal anti-Shc antibody. The immune complexes were precipitated with protein A-Sepharose 6MB and were washed three times with 50 m*M* Tris (pH 7.4) containing 2 m*M* sodium vanadate, and 0.1% Triton X-100.

Protein Analysis by Immunoblotting

After washing, the pellet was resuspended in Laemmli sample buffer *(40)* with 100 m*M* DTT and heated in a boiling water bath for 5 min. The samples were subjected to SDS-PAGE (10% polyacrylamide gels) in a Bio-Rad miniature slab gel apparatus. Electrotransfer of proteins from the gel to nitrocellulose was performed for 90 min at 120 V (constant) in a Bio-Rad miniature transfer apparatus (Mini-Protean) as described by Towbin et al. *(41)*. Nonspecific protein binding to the nitrocellulose was reduced by preincubating the filter overnight at 4ºC in blocking buffer (5% nonfat dry milk, 10 m*M* Tris, 150 m*M* NaCl, and 0.02% Tween-20). The prestained molecular mass standards used were phosphorylase B (105 kDa), bovine serum albumin (BSA) (82 kDa), ovalbumin (49 kDa), carbonic anhydrase (33.3 kDa), soybean trypsin inhibitor (28.6 kDa), and lysozyme (19.4 kDa). The nitrocellulose filter was incubated for 4 h at 22ºC with antiphosphotyrosine antibody (1 µg/mL), anti-Shc anti-body (1:100), or Grb2 antibody (1:100) diluted in blocking buffer (3% nonfat dry milk) and washed for 30 min in blocking buffer without nonfat dry milk. The blots

were then incubated with 2 μCi of [^{125}I]-Protein A (30 μCi/μg) in 10 mL of blocking buffer for 1 h at 22ºC and washed again. [^{125}I]-Protein A bound to the antibodies was detected by autoradiography using preflashed Kodak XAR film with Cronex Lightning Plus intensifying screens at −70ºC for 12–48 h. Band intensities were quantified by optical densitometry (model GS 300; Hoefer, San Francisco, CA) of the developed autoradiographs.

For Shc quantitation in either tissue, total extract samples (1 mg of protein/sample) were subjected to SDS-PAGE with no previous immunoprecipitation. After electrophoretic separation, proteins were transferred to nitrocellulose membranes and then blotted with specific antibody. Quantitative analysis was performed using scanning densitometry.

General Procedures

Protein quantification was performed using the Bradford dye method *(42)* and the Bio-Rad reagent with BSA as a standard. Plasma glucose levels were determined with a Beckman glucose analyzer (Beckman, Palo Alto, CA). Acute epinephrine-treated rats, streptozotocin-treated rats, and their respective controls underwent an iv insulin tolerance test (60 μg of insulin, injected into the portal vein). Samples were collected from the tail vein at 0 (basal), 4, 8, 12, and 16 min after hormone injection. Plasma glucose disappearance rate (K_{itt}) was obtained from the formula $0.693/t_{1/2}$. The plasma glucose $t_{1/2}$ was calculated from the slope of the least-square analysis of the plasma glucose concentrations during the linear phase of decline.

Statistical Analyses

Statistical analyses were based on the values obtained after densitometric analysis performed in the linear range of the film exposure. Comparisons of the Shc phosphorylation levels between controls (insulin-stimulated nontreated rats) vs diabetics and controls vs acute epinephrine-treated rats, as well as comparisons of the Shc/Grb2 association levels between controls vs diabetics and controls vs acute epinephrine-treated rats, were analyzed using the unpaired *t*-test. Basal (noninsulin-stimulated rats) levels of Shc phosphorylation and Shc/Grb2 association were determined by comparing normal and treated rats and were analyzed using the unpaired *t*-test. A p value ≤0.05 was considered significant.

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