Insulin Resistance and GLUT-4 Glucose Transporter in Adipocytes From Hypertensive Rats

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To investigate the mechanisms that cause insulin resistance in hypertension, experiments were performed to study the effect of insulin on glucose transport, GLUT-4 translocation from intracellular to plasma membranes and GLUT-4 phosphorylation in isolated adipocytes from normotensive Wistar (W) and spontaneously hypertensive rats (SHR). Glucose transport was measured in adipocytes incubated with 3-*O*-D[Methyl-^{3}H] glucose with and without insulin (0.1 to 5 nmol/L). GLUT-4 protein was determined by Western blot immunoanalysis with GLUT-4 antibody. Phosphorylation of GLUT-4 was measured by immunoprecipitation with GLUT-4 antibody followed by immunoanalysis with phosphoserine or phosphothreonine antibodies. Compared with adipocytes from W, insulin-stimulated glucose transport was lower in the SHR ($P < .05$). GLUT-4 protein expression was similar in adipocytes from W and SHR. Insulin increased GLUT-4 translocation from intracellular to plasma membranes in both groups. This effect was lower in the SHR ($P < .05$). The effect of insulin on GLUT-4 serine phosphorylation showed no changes in plasma membranes from W and decreased in the SHR ($P < .05$). In intracellular membranes, insulin increased specific GLUT-4 serine phosphorylation in both groups ($P < .05$), but the increase was lower in the SHR ($P < .05$). The results suggest that a deficient GLUT-4 translocation to plasma membranes in response to insulin shown in adipocytes from SHR, which was accompanied by a decrease in GLUT-4 phosphorylation at serine site, could be one of the causes of insulin resistance in hypertension.

ALTHOUGH INSULIN resistance is frequently observed in hypertension, the mechanisms involved are controversial. It has been proposed that the increase in peripheral resistance may induce insulin resistance by decreasing nutritional blood flow and, consequently, glucose delivery to peripheral tissues.[1-3] However, others have reported that decreasing blood pressure does not improve insulin sensitivity in both human subjects[4,5] and experimental animals.[6] In addition, we and other investigators have shown insulin resistance in isolated adipocytes from spontaneously hypertensive rats.[7,8] This latter finding argues against the hypothesis that vasoconstriction might be the cause of insulin resistance in hypertension.

The rate-limiting step in the uptake and metabolism of glucose by insulin target cells is glucose transport,[9] which is mediated by specific glucose transporters of the plasma membrane. In normal muscle cells and adipocytes, the glucose transporter isoform is GLUT-4, a 12-transmembrane domain protein that mediates transport of glucose in the direction of glucose gradient.[10,11] Insulin promotes GLUT-4 incorporation into plasma membrane, and this translocation from intracellular compartments appears to fail in the insulin resistance present in some form of diabetes.[12-15]

An increase of glucose transport by inhibiting the specific serine-threonine phosphatases PP1 and PP2A with okadaic acid has been reported.[16-20] Therefore, a phosphorylation event may be involved in the exocytic fusion of GLUT-4–containing vesicles with the plasma membrane. Two classes of serine/threonine kinases known to act downstream of phosphatidylinositol (PI)3-kinase, namely Akt or PKB, and the atypical PKC isoforms ξ and λ were implicated in the insulin-stimulated GLUT-4 translocation.[21-24] Despite the fact that these studies demonstrate that PI3-kinase function is a necessary event, several lines of evidence have demonstrated that this is not sufficient and that insulin must generate additional signals that function in conjunction with PI3-kinase.[25-29]

GLUT-4 phosphorylation induced by different agonists in adipocytes prelabeled with $^{32}P_i$ was unrelated to an increase in glucose transport.[30-32] In this report, we are presenting evidence that insulin-stimulated GLUT-4 translocation is decreased in adipocytes from hypertensive animals. This impaired GLUT-4 translocation could be related to a deficient GLUT-4 phosphorylation at serine site.

MATERIALS AND METHODS

Animals

Male spontaneously hypertensive (SHR) (5-months-old) and age-matched normotensive Wistar (W) rats originally from Charles River Breeding Laboratories (Wilmington, MA) and bred in our institution were used throughout this study. The study was performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Animal body weight (BW) and blood pressure were determined once a week. Systolic blood pressure (BP) was measured with the indirect tail-cuff technique.[33] Rats were killed by decapitation and the heart and epididymal fat pads were removed. Atria and all adjacent connective tissue were removed, and the remaining tissue was blotted and weighed to determine heart weight (HW). The ratio between HW in milligrams and BW weight in grams was taken to measure hypertrophy. Epididymal fat pads were used to isolate adipocytes.

Adipocytes

Isolated adipocytes were obtained by digestion of epididymal fat pad with collagenase (Worthington, Freehold, NJ).[34] Krebs Ringer-3-[(N-morpholino) propanesulfonic acid] (KRM) at pH 7.4 containing 4%

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bovine serum albumin (BSA, Fraction V, Gibco BRL, Grand Island, NY) was used as incubation buffer.

Glucose Transport

Glucose transport was determined as previously described.[7] Briefly, pooled adipocytes from 3 rats were suspended (10^6 cells/mL) in KRM at pH 7.4 with 2% BSA and 2 mmol/L pyruvate. Aliquots of the cell suspension were preincubated at 37°C for 30 minutes, followed by a 10-minute incubation with and without 0.1 to 5 nmol/L insulin. Glucose transport was initiated by addition of 0.1 mmol/L 3-O-D-[Methyl-^{3}H] glucose (42 mCi/mmol, New England Nuclear, Boston, MA). After 8 seconds, uptake was stopped with 400 μL 1 mmol/L phloretin. Aliquots of the cell suspension were pipetted into microcentrifuge tubes containing silicone oil (Thomas Scientific, Swedesboro, NJ) and centrifuged for 30 secomds at 10,000 $\times$ *g*. The tubes were cut through the oil layer, and the radioactivity associated with the cells was measured. Noncarrier-mediated uptake was assessed in parallel incubations with 400 μmol/L phloretin and the value subtracted from each determination. The assay was performed in triplicate and the results were averaged. Results were expressed as nanomoles of 3-*O*-D[Methyl-^{3}H]glucose/10^5 cells per 8 seconds.

Subcellular Fractionation of Adipocytes

To study the effect of insulin on subcellular distribution and phosphorylation of fat cell GLUT-4, adipocytes from 3 to 6 rats were prepared. The fat cells were suspended in KRM 4% BSA at pH 7.4 and incubated as described for glucose transport with and without 5 nmol/L insulin. Plasma membrane-enriched fraction (PM) and intracellular membranes (ICM) were prepared by a previously characterized procedure.[35] Adipocytes were homogenized in Buffer A (in mmol/L: 250 sucrose, 20 Tris, 1.2 EGTA, 20 β-mercaptoethanol containing 1 phenylmethylsulfonyl fluoride [PMSF], 4 leupeptin, 0.5 aprotinin as protease inhibitors, and 1 $Na_2P_20_7$, 1 NaF to inhibit phosphatases). The homogenate was centrifuged at 3,000 $\times$ *g* for 15 minutes. The infranate was centrifuged for 30 minutes at 20,000 $\times$ *g* to obtain PM, and the supernatant was centrifuged at 400,000 $\times$ *g* for 60 minutes to obtain ICM. Membrane fractions were resuspended in buffer B (in mmol/L: 250 sucrose, 20 Tris, 1 EDTA, 25 NaF, 10 $Na_2P_20_7$ and protease inhibitors). Protein was determined by the method of Bradford.[36]

Immunoprecipitation of GLUT-4

Subcellular membrane fractions were treated with lysis buffer consisting of 1% Triton X-100, 100 mmol/L NaCl in buffer B and incubated with 30 μL/mL protein A Sepharose (8% wt/vol in buffer B) for 1 hour. The protein A Sepharose was pelleted at 10,000 $\times$ *g* for 1 minute and the supernatant was incubated overnight with 2 μg/mL polyclonal GLUT-4 antibody (Biogenesis, Kingston, NH). After this, 1% BSA and 30 μL/mL protein A Sepharose were added, and the incubation was continued for an additional 2 hours. The protein A Sepharose-antigen-antibody pellets were separated by centrifugation and washed twice with lysis, once with 0.1% TritonX-100, 50 mmol/L Tris, 300 mmol/L NaCl, 5 mmol/L EDTA, 0.02% azide and once with 50 mmol/L Tris pH 6.8.

Immunoanalysis by Western Blot

Polyacrylamide-gel electrophoresis (PAGE) of fat cell membranes or Protein A-antigen-antibody pellets obtained by immunoprecipitation of fat cell membranes was performed on 10% sodium dodecyl sulfate (SDS)-PAGE. The samples were solubilized by addition of sample buffer containing 125 mmol/L Tris-HCl pH 6.8, 8% SDS, 40% glycerol, 20% β-mercaptoethanol with bromophenol blue as a tracking dye. The separated proteins were transferred to polyvinylidene difluoride membranes (PVDF, Millipore Bedford, MA). Immunoanalysis was performed with rabbit polyclonal antibody to GLUT-4 (Biogenesis) mouse antiphosphoserine (Chemicon, Temecula, CA) or antiphosphothreonine (Santa Cruz, Santa Cruz, CA) antibodies as applied. The blots were developed by incubation with a polyclonal goat antirabbit or antimouse immunoglobulin coupled to peroxidase (Santa Cruz) and enhanced chemiluminescence. The membranes were exposed to Kodak (Rochester, NY) X-OMAR AR and the autoradiographies analyzed with an Ultroscan XL densitometer (Pharmacia, Uppsala, Sweden). The results were expressed in arbitrary units.

Table 1. General Characteristics of W and SHR Rats

Animals	BP (mm Hg)	BW (g)	HW (mg)	HW/BW (mg/g)
W (n = 60)	120.5 ± 1.0	321.8 ± 9.5	814 ± 29	2.53 ± 0.05
SHR (n = 60)	181.2 ± 4.1*	267.5 ± 6.0*	898 ± 24*	3.36 ± 0.09*

Abbreviations: W, Wistar; SHR, spontaneously hypertensive rats; BP, systolic blood pressure; BW, body weight; HW, heart weight.
*$P < .05$ *v* W (*t* test).

Statistics

The *t* test (Table 1), 2-way analysis of variance (ANOVA) (Fig 1), and 1-way ANOVA with Bonferroni posttest (Figs 2, 3, and 4) were used to analyze the results. $P < .05$ was considered statistically significant.

RESULTS

General characteristics of the W and SHR groups are shown in Table 1. Systolic BP, HW, and cardiac hypertrophy (HW/BW) were significantly elevated in SHR compared with W rats ($P < 0.05$). Although BW was lower in the SHR ($P < .05$), the epididymal fat pad weights were similar in both groups of animals.

Insulin-stimulated glucose transport in adipocytes from W and SHR rats is shown in Fig 1. The results from 5 experiments show that compared with normotensive rats glucose transport in adipocytes from the SHR was significantly impaired. Maximal glucose uptake in adipocytes from W and SHR groups was 0.63 ± 0.05 and 0.35 ± 0.03 nmol/10^5cells, respectively ($P < .05$). Basal glucose transport was similar in adipocytes from W (0.11 ± 0.02) and SHR (0.13 ± 0.02 nmol/10^5 cells). Insulin sensitivity determined as the concentration of insulin required to reach half-maximal effect on glucose transport (EC_{50}) was the same in both groups of animals. EC_{50} = 0.10 ± 0.04 and 0.11 ± 0.03 nmol/L in normotensive and hypertensive rats, respectively.

The effect of insulin on subcellular distribution of the GLUT-4 glucose transporter isoform was next analyzed. The autoradiography from 1 experiment in Fig 2A shows the distribution of GLUT-4 between PM and ICM from W and SHR adipocytes incubated during 10 minutes in the absence and presence of 5 nmol/L insulin. The overall results from 5 experiments in Fig 2B show that in the absence of insulin, GLUT-4 is mainly localized in ICM. This distribution, as well as the amount of GLUT-4 protein expression in PM and ICM, were similar in adipocytes from W and SHR. The same expression of GLUT-4 protein in PM from W and SHR is in agreement with the same basal glucose transport observed in adipocytes from both groups.

Figure 2A shows that insulin induced an increase of GLUT-4

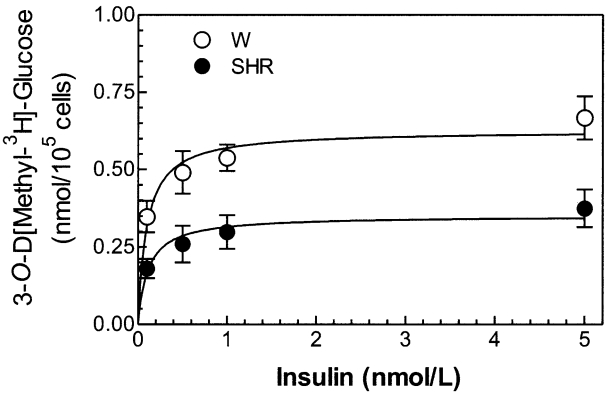

Fig 1. Effect of insulin on glucose transport by isolated adipocytes from W and SHR rats. Adipocytes were incubated with and without insulin (0.1 to 5 nmol/L) as described in Materials and Methods. Basal glucose transport was subtracted from each value in the presence of insulin. Results are the means ± SE of 5 experiments performed in triplicate. Insulin-stimulated glucose transport was lower in the SHR ($P < .05$, 2-way ANOVA).

in PM and a decrease in ICM in adipocytes from both strains. However, the effect of insulin promoting the translocation of GLUT-4 from ICM to PM was lower in the SHR. The results from 5 experiments are shown in Fig 2C. Compared with adipocytes incubated under basal conditions, insulin elicited a 93.3% ± 15.0% increase of GLUT-4 in PM from W, whereas in the SHR, the increase was significantly lower (42.7% ± 13.2%, $P < .01$). On the other hand, the decrease of GLUT-4 induced by insulin in ICM was 60.0% ± 3.3% in W and 20.0% ± 5.1% in the SHR ($P < .05$).

Because phosphorylation events have been implicated in GLUT-4 translocation in response to different stimuli,[16-20] we next analyzed the effect of insulin on GLUT-4 phosphorylation at serine and threonine sites in adipocytes from normotensive and hypertensive rats. Subcellular membrane fractions were immunoprecipitated with GLUT-4 antibody and immunoanalyzed in duplicate by Western blot. One blot was probed with specific phosphoserine (PhSer) or phosphothreonine (PhThr) antibody and the other one with GLUT-4 antibody. The autoradiography from 1 experiment in Fig 3A shows the effect of insulin on GLUT-4 phosphorylation (PhSer-GLUT-4) and GLUT-4 protein distribution between PM and ICM. The results from 5 experiments showing PhSer-GLUT-4 are depicted in Fig 3B and Fig 3C. Basal phosphorylation in PM (Fig 3B) and ICM (Fig 3C) was the same in normotensive and hypertensive rats. In the presence of insulin, Fig 3B shows an increase of PhSer-GLUT-4 in PM from W from 2.20 ± 0.34 to 4.42 ± 0.32 arbitrary units ($P < .05$), without significant changes in PM from the SHR (2.07 ± 0.48 and 2.30 ± 0.15 arbitrary units, respectively. In ICM (Fig 3C), insulin increased PhSer-GLUT-4 in both groups. In W from 2.23 ± 0.18 to 3.21 ± 0.28 ($P < .05$) and in the SHR from 2.01 ± 0.21 to 4.34 ± 0.20 arbitrary units ($P < .05$). This effect of insulin on GLUT-4 phosphorylation in ICM from the SHR was higher than in W ($P < .05$).

Negligible phosphorylation of GLUT-4 at threonine site was observed, and it was not affected by incubation with insulin (not shown).

Because insulin induces changes in subcellular distribution of GLUT-4 (see Fig 2), GLUT-4 phosphorylation relative to GLUT-4 protein was calculated by the ratio between the area values of PhSer-GLUT-4 peaks to those of GLUT-4 protein taken from the experiments described in Fig 3. Figure 4 shows that insulin did not change the ratio PhSer-GLUT-4/GLUT-4 in PM from W rats, which means that the effect of insulin increasing GLUT-4 translocation from ICM fraction to PM was accompanied by a concomitant increase in PhSer-GLUT-4. In PM from the SHR, the ratio was decreased by insulin indicating that the lower translocation of GLUT-4 in response to insulin in the SHR shown in Fig 2 was also accompanied by a lower serine phosphorylation of GLUT-4. In ICM, insulin increased the ratio PhSer-GLUT-4/GLUT-4 in both groups. However,

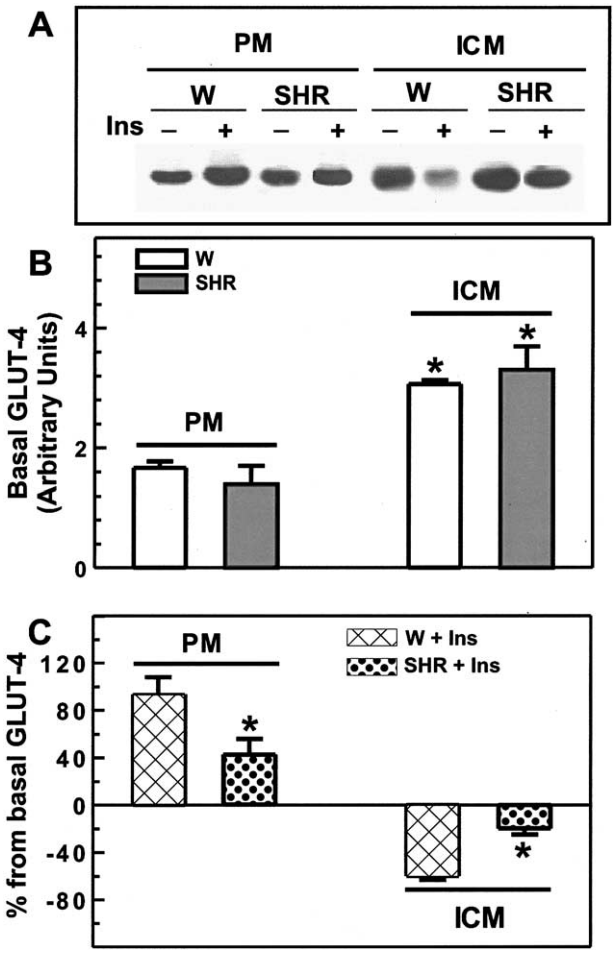

Fig 2. Effect of 5 nmol/L insulin on GLUT-4 distribution between PM and ICM in adipocytes from W and SHR rats. (A) Immunoblot from 1 experiment probed with GLUT-4 antibody. (B) Results from 5 experiments show in arbitrary units the distribution of GLUT-4 between PM and ICM from adipocytes of W and SHR incubated without insulin, *$P < .05$ *v* PM (1-way ANOVA). (C) Effect of 5 nmol/L insulin expressed as % increase from basal GLUT-4 in PM and as % decrease from basal GLUT-4 in ICM from adipocytes of both groups. *$P < .05$ compared with W (1-way ANOVA). Results are the means ± SE.

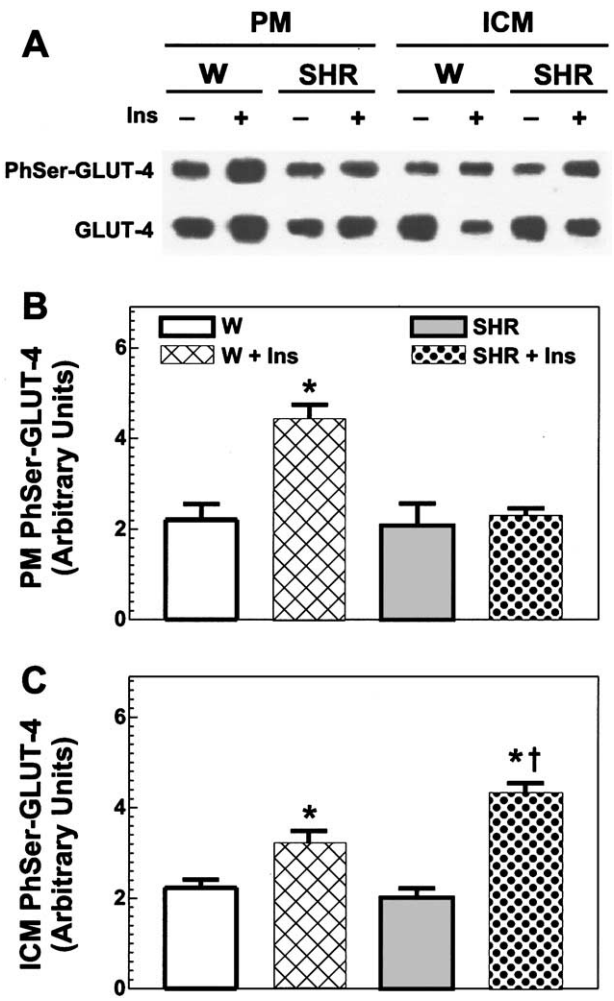

Fig 3. Effect of 5 nmol/L insulin on PhSerGLUT-4 and GLUT-4 protein in PM and ICM from adipocytes of W and SHR rats. Subcellular membrane fractions were immunoprecipitated with GLUT-4 antibody and immunoanalyzed in duplicate by Western blot. One blot was probed with specific PhSer antibody and the other one with GLUT-4 antibody. (A) Immunoblots from 1 experiment probed with antiphosphoserine and anti–GLUT-4 antibodies, respectively. Results from 5 experiments are shown in (B) and (C). (B) PhSerGLUT-4 in PM from W and SHR adipocytes incubated without and with insulin, *$P < .05$ *v* basal (1-way ANOVA). (C) PhSerGLUT-4 in ICM from W and SHR adipocytes incubated without and with insulin, *$P < .05$ compared with W and SHR without insulin, respectively; †$P < .05$ *v* W plus insulin (1-way ANOVA). Results are the means ± SE.

the effect of insulin was lower in the SHR ($P < .05$) compared with W.

The overall results indicate that the diminished effect of insulin on glucose transport in the SHR is accompanied with a deficient translocation of GLUT-4 from intracellular compartments to PM. Similar GLUT-4 protein expression was obtained in adipocytes from W and SHR. In addition, compared with adipocytes from normotensive animals, a decrease of PhSer-GLUT-4 in response to insulin in PM and ICM from the SHR was also observed.

DISCUSSION

In this study, the effect of insulin on glucose transport, GLUT-4 translocation from intracellular compartments to PM and GLUT-4 phosphorylation at serine site in adipocytes from normotensive and hypertensive rats were compared.

Under basal conditions glucose transport in adipocytes from W and SHR rats was similar, but the maximal glucose uptake in response to insulin was lower in the SHR without changes in insulin sensitivity. These data are in accordance with our previously reported results.[7] Glucose transport across PM seems to be the rate-limiting step of glucose metabolism.[9] GLUT-4 is recycled between PM and intracellular storage pools, and the steady-state distribution of GLUT-4 under basal conditions favors intracellular compartments over PM.[11,37,38] These findings have been confirmed in the present study. Our data show that GLUT-4 protein expression under basal conditions in PM and ICM was not different in adipocytes from normotensive and hypertensive rats. This is consistent with the same basal glucose transport in both groups of animals. Moreover, the decrease in insulin-stimulated glucose uptake in adipocytes from SHR is not caused by a deficient GLUT-4 protein expression. In other models of insulin resistance, such as obesity[39] and type 2 diabetes,[40] a significant reduction in GLUT-4 protein expression in adipose tissue was reported. In the presence of insulin, the equilibrium of the recycling process is changed in favor of the translocation of GLUT-4 from intracellular storage sites to PM.[41,42] This effect of insulin on GLUT-4 translocation was impaired in adipocytes from hypertensive rats.

In experiments performed with adipocytes incubated in the presence of okadaic acid to inhibit serine-threonine phosphatases, it has been proposed that insulin increases PM GLUT-4 level largely by preventing GLUT-4 internalization as a result of GLUT-4 dephosphorylation.[16,18,20] According to our results, specific GLUT-4 serine phosphorylation in response to insulin

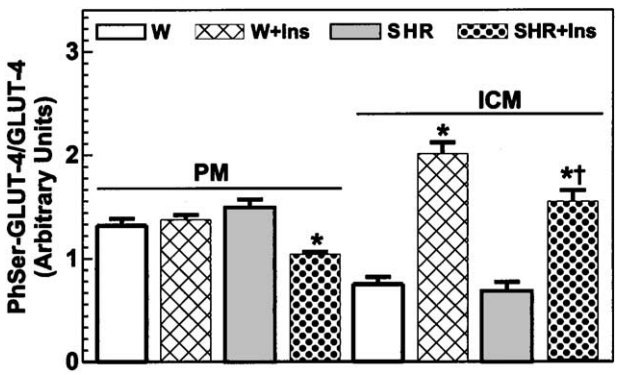

Fig 4. From the experiments described in Fig 3, the effect of 5 nmol/L insulin on the specific GLUT-4 phosphorylation (PhSerGLUT-4/GLUT-4) in PM and ICM from W and SHR adipocytes was calculated. Compared with basal value, insulin did not change specific PhSerGLUT-4 in PM from W and decreased in PM from SHR ($P < .05$). In ICM from both groups, insulin increased specific PhSerGLUT-4 compared with basal ($P < .05$,) but this increase was lower in the SHR. †$P < .05$ compared with W plus insulin (1-way ANOVA). Results are the means ± SE.

was unchanged in PM from adipocytes of normotensive animals (see Fig 4); ie, the increase in PM GLUT-4 in response to insulin was accompanied by a simultaneous increase in serine-phosphorylation. This effect of insulin was not observed in adipocytes from hypertensive animals, because insulin decreased specific GLUT-4 serine-phosphorylation in PM. In ICM, insulin stimulated specific GLUT-4 serine-phosphorylation in adipocytes from both strains, but this effect was lower in the SHR (Fig 4). These results suggest that GLUT-4 phosphorylation at serine site is required for GLUT-4 translocation to PM, and that the diminished translocation of GLUT-4 found in the SHR was due to a deficient GLUT-4 serine-phosphorylation.

Under similar conditions, phosphorylation of GLUT-4 at threonine site was scanty and unaffected by insulin. This negative result reinforces the proposal that phosphorylation at serine residue is necessary to increase GLUT-4 translocation in response to insulin. Our results are in disagreement with previous studies in which Glut-4 phosphorylation was not considered to be an attribute for insulin to stimulate glucose transport.[25,26] However, those experiments were conducted under different experimental conditions, among others, to measure total phosphorylation in $^{32}P_i$-labeled adipocytes, which did not allow detecting GLUT-4 specific phosphorylation at serine site.

The major contribution of this study is to present the first evidence of decreased GLUT-4 translocation from intracellular compartments to PM in response to insulin in isolated adipocytes from hypertensive animals. We show that this was not caused by deficient GLUT-4 protein expression. Rather, our data suggest that this may have been due to impaired serine phosphorylation. However, additional study will be needed to identify a specific mechanism for the decreased serine phosphorylation.

We would like to emphasize that we cannot rule out completely a possible role played by vasoconstriction to the insulin resistance detected in hypertension. However, we describe a mechanism that could be, at least, one of the causes of insulin resistance in hypertension.

REFERENCES

1. Baron AD, Brechtel-Hook G, Johnson A, et al: Skeletal muscle blood flow. A possible link between resistance and blood pressure. Hypertension 21:129-135, 1993

2. Egan BM: Neurohumoral, hemodynamic and microvascular changes as mechanisms of insulin resistance in hypertension: A provocative but partial picture. Int J Obes 15:133-139, 1991

3. Julius S, Gudbrandsson T, Jamerson K, et al: The hemodinamic link between insulin resistance and hypertension. Hypertension 9:983-986(hypothesis), 1991

4. Fogari R, Zoppi A, Lazzari P, et al: ACE inhibition but not angiotensin II antagonism reduces plasma fibrinogen and insulin resistance in overweight hypertensive patients. J Cardiovasc Pharmacol 32:616-620, 1998

5. Laakso M, Karjalainen L, Lempiäinen-Kuosa P: Effects of losartan on insulin sensitivity in hypertensive subjects. Hypertension 28: 392-396, 1996

6. Tomiyama H, Kushiro T, Abeta H, et al: Kininins contribute to the improvement of insulin sensitivity during treatment with angiotensin converting enzyme inhibitor. Hypertension 23:450-455, 1994

7. Caldiz CI, Chiappe de Cingolani GE: Insulin resistance in adipocytes from spontaneously hypertensive rats: Effect of long-term treatment with enalapril and losartan. Metabolism 48:1041-1046, 1999

8. Reaven GM, Chang H, Hoffman BB, et al: Resistance to insulin-stimulated glucose uptake in adipocytes isolated from spontaneously hypertensive rats. Diabetes 38:1155-1160, 1989

9. Crofford OB, Renold AE: Glucose uptake by incubated rat epididymal adipose tissue. Rate-limiting steps and site of insulin action. J Biol Chem 240:24-21, 1965

10. Barret MP, Walmsley AR, Gould GW: Structure and function of facilitative sugar transporters. Curr Opin Cell Biol 11:496-502, 1999

11. Douen AG, Ramlal T, Rastogi S, et al: Exercise induces recruitment of the "insulin-responsive glucose transporter." Evidence for distinct intracellular insulin and exercise-recruitable transporter pools in skeletal muscle. J Biol Chem 265:13427-13430, 1990

12. Garvey WT, Maianu L, Zhu JH, et al: Evidence of defects in the trafficking and translocation of GLUT-4 glucose transporters in skeletal muscle as a cause of human insulin resistance. J Clin Invest 101:21377-2386, 1998

13. King PA, Horton ED, Hirshman MF, et al: Insulin resistance in obese Zucker rat (fa/fa) skeletal muscle is associated with a failure of glucose transporter translocation. J Clin Invest 90:1568-157, 1992

14. Klip A, Ramlal T, Bilan PJ, et al: Recruitment of GLUT-4 glucose transporters by insulin in diabetic rat skeletal muscle. Biochem Biophys Res Commun 172:728-736, 1990

15. Zierath JR, He L, Gumà A, et al: Insulin action on glucose transport and plasma membrane GLUT-4 content in skeletal muscle from patients with NIDDM. Diabetologia 39:1180-1189, 1996

16. Corvera S, Jaspers S, Pasceri M: Acute inhibition of insulin-stimulated glucose transport by the phosphatase inhibitor, okadaic acid. J Biol Chem 266:9271-9275, 1991

17. Haystead TAJ, Sim TAR, Carling D, et al: Effects of the tumor promoter okadaic acid on intracellular protein phosphorylation and metabolism. Nature 337:78-81, 1989

18. Lawrence JC Jr, Hiken JF, James DE: Stimulation of glucose transport and glucose transporter phosphorylation by okadaic acid in rat adipocytes. J Biol Chem 265:19768-19776, 1990

19. Rampall AJ, Jhun BH, Kim S, et al: Okadaic stimulates glucose transport in rat adipocytes by increasing the externalization rate constant of GLUT-4 recycling. J Biol Chem 270:3938-3943, 1995

20. Tanti JF, Gremeaux T, Van Obberghen E, et al: Effects of okadaic acid, an inhibitor of protein phosphatases-1 and -2A, on glucose transport and metabolism in skeletal muscle. J Biol Chem 266:2099-2103, 1991

21. Cong LN, Chen H, Li Y, et al: Physiological role of Akt in insulin-stimulated translocation of GLUT-4 in transfected rat adipose cells. Mol Endocrinol 11:1881-1890, 1997

22. Kohn AD, Summers SA, Birnbaum SA, et al: Expression of a constitutively active Akt ser/thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. J Biol Chem 271:31372-31378, 1996

23. Kohn AD, Barthel A, Kovacina KS, et al: Construction and characterization of a conditionally active version of the serine/threonine kinase Akt. J Biol Chem 273:11937-11943, 1998

24. Kotani K, Ogawa W, Matsumoto M, et al: Requirement of atypical protein kinase lambda for insulin stimulation of glucose uptake but not for Akt activation in 3T3-L1 adipocytes. Mol Cell Biol 18: 6971-6982, 1998

25. Isakoff SJ, Taha C, Rose E, et al: The inability of phosphatidylinositol 3-kinase activation to stimulate GLUT-4 translocation indicates additional signaling pathways are required fro insulin-stimulated glucose uptake. Proc Natl Acad Sci USA 92:10247-10251, 1995

26. Krook A, Whitehead JP, Dobson SP, et al: Two naturally oc-

curring insulin receptor tyrosine kinase domain mutants provide evidence that phosphoinositide 3-kinase activation alone is not sufficient for the mediation of insulin's metabolic and mitogenic effects. J Biol Chem 272:30208-30214, 1997

27. Guilherme A, Czech MP: Stimulation of IRS-1-associated phosphatidylinositol 3-kinase and Akt/protein kinase B but not glucose transport by beta 1-integrin signaling in rat adipocytes. J Biol Chem 273:33119-33122, 1998

28. Staubs PA, Nelson JG, Reichart DR, et al: Platelet-derived growth factors inhibit insulin-stimulation of insulin receptor substrate-1-associated phosphatidyl-inositol 3-kinase in 3T3-L1 adipocytes without affecting glucose transport. J Biol Chem 273:25139-25147, 1998

29. Jiang T, Sweeney G, Rudolf MT, et al: Membrane permeant esters of phosphatidylinositol 3,4,5-triphosphaste. J Biol Chem 273: 11017-11024, 1998

30. James DE, Hiken J, Lawrence JC: Isoproterenol stimulates phosphorylation of the insulin-regulatable glucose transporter in rat adipocytes. Proc Natl Acad Sci USA 86:8368-8372, 1989

31. Nishimura H, Saltis J, Habberfield AD, et al: Phosphorylation state of the GLUT-4 isoform of the glucose transporter in subfractions of the rat adipose cell: Effects of insulin, adenosine, and isoproterenol. Proc Natl Acad Sci USA 88:11500-11504, 1991

32. Reuch JEB, Sussman KE, Draznin B: Inverse relationship between GLUT-4 phophorylation and its intrinsic activity. J Biol Chem 268:3348-3351, 1993

33. Buñag RA: Vasodilation in awake rats of a tail-cuff method for measuring systolic pressure. J Appl Physiol 34:279-282, 1973

34. Rodbell M: Metabolism of isolated fat cells I. Effects of hormones on glucose metabolism and lipolysis. J Biol Chem 239:375-380, 1964

35. Chiappe de Cingolani GE, Goers JWF, Giannotti M, et al: Comparative effects of insulin and isoproterenol on lipoprotein lipase in rat adipose cells. Am J Physiol 270:C1461-C1467, 1996

36. Bradford MM: Rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-254, 1976

37. Hirshman MF, Goodyear LJ, Wardzala LJ, et al: Identification of an intracellular pool of glucose transporters from basal and insulin-stimulated rat skeletal muscle. J Biol Chem 265:987-991, 1990

38. Ramlal T, Saravia V, Bilan PJ, et al: Insulin-mediated translocation of glucose transporters from intracellular membranes to plasma membranes: Sole mechanism of stimulation of glucose transport in L6 muscle cells. Biochem Biophys Res Commun 157:1329-1335, 1988

39. Garvey WT, Maianu L, Hueckstead TP, et al: Pretranslational suppression of a glucose transporter protein causes insulin resistance in adipocytes from patients with non-insulin dependent diabetes mellitus. J Clin Invest 87:1072-1081, 1991

40. Sinha MK, Raineri-Maldonado C, Buchanan C, et al: Adipose tissue glucose transporters in NIDDM: Decreased levels of the muscle/fat isoform. Diabetes 40:472-477, 1991

41. Cushman SW, Wardzala LJ: Potential mechanisms of insulin action on glucose transport in the isolated rat adipose cell. J Biol Chem 255:4758-4762, 1989

42. Watson RT, Pessin JE: Subcellular compartmentalization and trafficking of the insulin-responsive glucose transport, GLUT-4. Exp Cell Res 271:75-83, 2001