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Pharmacological inhibition of p38 MAP kinase results in improved glucose uptake in insulin-resistant 3T3-L1 adipocytes

Christian J. Carlson, Cristina M. Rondinone*

Metabolic Disease Research, Global Pharmaceutical Research and Development, Abbott Laboratories, IL 60064, USA

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Abstract

Inhibition of p38, a member of the mitogen-activated protein kinase family, has been shown to prevent the loss of GLUT4 protein expression in insulin-resistant adipocytes without improving insulin receptor substrate 1 (IRS-1) protein levels and presumably insulin signaling. Thus, it was unclear whether p38 inhibitors would have a beneficial effect upon insulin-stimulated glucose uptake. We evaluated the effects of p38 inhibition during the development of insulin resistance upon glucose uptake and components of the insulin signaling pathway to determine the therapeutic value of p38 inhibitors. Treatment with the specific p38 inhibitor, A304000, during the development of insulin resistance increased basal glucose uptake as well as glucose uptake in response to a subsequent insulin stimulation. p38 inhibition increased GLUT1 protein levels and prevented the loss of GLUT4. However, p38 inhibition did not prevent the loss of IRS-1 protein levels or insulin signaling to PKB in insulin-resistant cells. Rapamycin, an inhibitor or mTOR, could partially improve insulin-stimulated glucose uptake through maintaining IRS-1 protein levels. Combined treatment with both A304000 and rapamycin had an additive effect upon glucose uptake. These data indicate that p38 inhibition can enhance glucose uptake through regulating the expression of GLUT1 and 4, but did not prevent the development of insulin resistance.

1. Introduction

Insulin-mediated glucose uptake is compromised in skeletal muscle and adipose tissue of insulin-resistant and diabetic patients. In adipose tissue, decreased insulin-mediated glucose uptake is the result of decreased protein levels of the insulin-responsive glucose transporter (GLUT4) and insulin receptor substrate 1 (IRS-1) [1-3]. GLUT4 is the primary insulin-responsive glucose transporter in adipose tissue and is necessary for insulin-stimulated glucose uptake [4]. In human beings, GLUT4 protein levels are decreased in adipose tissue of type 2 diabetics as well as insulin-resistant first-degree relatives [5,6]. Animals with diminished levels of GLUT4, through heterozygous gene ablation, are insulin resistant and develop diabetes and many of the associated complications [7]. Furthermore, when GLUT4 is knocked out specifically in adipose tissue, the animals develop insulin resistance in both skeletal muscle and liver [8]. Thus, the loss of GLUT4, specifically in adipose tissue, is an important factor in the development of insulin resistance and diabetes.

P38 is a member of the mitogen-activated protein kinase family of serine/threonine kinases. Like other members of the mitogen-activated protein kinase family, p38 is activated by dual phosphorylation on tyrosine and threonine residues as a result of activation of an upstream signaling cascade. P38 is capable of regulating many cellular processes including cell morphology as well as expression of numerous target genes. P38 is necessary for the full differentiation of preadipocytes into adipocytes [9,10] and contributes to insulin-mediated glucose uptake in muscle cells and adipocytes [11,12]. Pharmacological inhibition of p38 can prevent the full differentiation of adipocytes, whereas constitutive activation can promote differentiation [9,10]. Treatment of adipocytes or muscle cells with p38 inhibitors can diminish the glucose uptake activity of GLUT4 in response to insulin, without affecting the translocation of GLUT4 to the cell membrane, suggesting that p38 is involved with a yet to be identified activation step [11,12].

* Corresponding author. Metabolic Disease Research, Hoffman La Roche, Inc, 340 Kingsland St, Nutley, NJ 07110, USA. Tel.: +1 718 430 2853.

E-mail address: cristina.rondinone@roche.com (C.M. Rondinone).

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We have recently discovered that p38 is inappropriately activated in adipocytes from type 2 diabetic patients [13]. Subsequent research demonstrated that pharmacological inhibition of p38 was capable of preventing the loss of GLUT4 protein expression in a cellular model of insulin resistance. In addition, constitutive activation of the p38 pathway down-regulated the expression of GLUT4 in adipocytes, resulting in diminished insulin-stimulated glucose uptake [14]. Thus, p38 is an important step in the down-regulation of GLUT4 expression, indicating that p38 inhibitors may be of therapeutic value. Although p38 inhibitors improved GLUT4 protein levels, they had no effect upon the loss of IRS-1 and thus presumable did not improve insulin signaling. Furthermore, p38 may also be an important regulator of insulin-stimulated glucose uptake through activation of GLUT4. Thus, it was unclear whether p38 inhibitors would have a beneficial effect in insulin-resistant adipocytes. We wished to determine if pharmacological inhibition of p38 would improve insulin-stimulated glucose uptake in adipocytes to better understand the therapeutic value of p38 in the treatment of insulin resistance.

In the current series of experiments, we demonstrate that inhibition of p38 during the development of insulin resistance resulted in a partial recovery of insulin-stimulated glucose uptake, which was associated with increased protein levels of both GLUT1 and GLUT4. The effects of p38 inhibition were limited to the expression of glucose transporters, as treatment with the p38 inhibitor did not improve insulin signaling through IRS-1, PI 3 kinase, or protein kinase B (PKB); thus, glucose uptake can be improved without an improvement in insulin signaling. Subsequently, we wished to determine if recovery of insulin signaling in addition to the recovery of GLUT4 would further improve glucose uptake. Both basal and insulin-stimulated glucose uptake were further increased when cells were treated with the p38 inhibitor and rapamycin, an inhibitor of mammalian target of rapamycin (mTOR) that has been shown to recover insulin signaling. Thus, inhibition of p38 during the development of insulin resistance has a beneficial effect upon insulin-stimulated glucose uptake, suggesting that p38 inhibitors may be of therapeutic value in the treatment of insulin resistance and diabetes.

2. Experimental design and methods

2.1. 3T3-L1 cell culture

3T3-L1 cells were grown and maintained as fibroblasts in Dulbecco's modified Eagle's medium (DMEM, Life Technologies, Gaithersburg, Md) with high glucose containing 10% fetal bovine serum (FBS, HyClone, Logan, Utah) in a humidified atmosphere composed of 95% air and 5% CO_2. Three days after plating, cells were differentiated into adipocytes by exposure to DMEM high glucose with 10% FBS, 0.4 μg/mL dexamethasone, 0.5 mmol/L isobutylmethylxanthine, and 5 μg/mL insulin (Sigma, St Louis, Mo). After an additional 3 days, the media were changed and the cells were maintained with DMEM containing 10% FBS.

2.2. Induction of insulin resistance

Fully differentiated day 12 adipocytes were used for all experiments. Insulin resistance was induced by incubation with insulin for 20 hours as described previously [13] (Fig. 1). Briefly, cells were starved for 2 hours in DMEM/0.5% bovine serum albumin (BSA, Life Technologies) before treatment with vehicle (dimethyl sulfoxide [DMSO], Sigma), A304000 (Abbott Laboratories, Abbott Park, Ill) [12,13], or rapamycin (Alexis, San Diego, Calif) for 30 minutes before incubation with insulin (1 μmol/L) for 20 hours. After 20 hours, cells were washed extensively in DMEM/0.5% BSA and starved for an additional 2 hours. Subsequently, media were changed to DMEM without glucose (Life Technologies) for 30 minutes before a submaximal stimulation with insulin (10 nmol/L). Cells were then used for measurement of glucose uptake or preparation of whole cell lysates.

2.3. Measurement of glucose uptake

After the 18-hour chronic incubation with insulin, cells were washed 3 times with DMEM/0.5% BSA and starved again for 2 hours. Cells were then incubated for 30 minutes in glucose-free DMEM before an acute stimulation with insulin (10 nmol/L). After 30 minutes, ^{3}H 2-deoxyglucose (1 μCi/reaction) (Amersham, Arlington Heights, Ill) in the presence of glucose (182 μmol/L final concentration) was added to the media for an additional 30 minutes. Cells were rinsed with cold phosphate-buffered saline (PBS) and solubilized with 0.1N NaOH. Incorporation of labeled 2-deoxyglucose was measured by scintillation counting on a Beckman Scintillation counter.

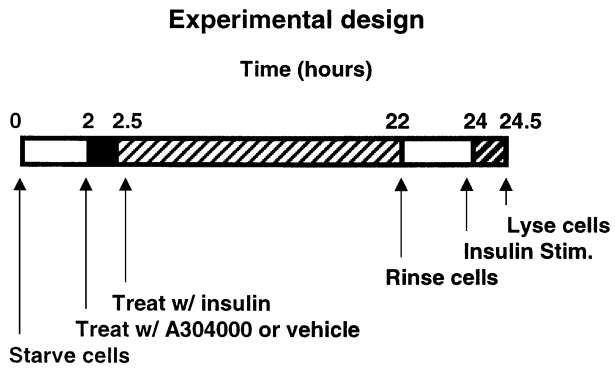

Fig. 1. Experimental design—Fully differentiated (day 12) 3T3-L1 adipocytes were starved for 2 hours in DMEM with 0.5% BSA. Cells were then treated with p38 inhibitor (A304000) or vehicle (DMSO) for 30 minutes before treatment with 1 μmol/L concentration of insulin for 20 hours. Subsequently, cells were rinsed in PBS and restarved for 2 hours. Cells were stimulated with submaximal dose of insulin (10 nmol/L) for 5 or 30 minutes before analysis.

2.4. Preparation of cellular lysates

After a 5-minute submaximal insulin stimulation, media were aspirated from the cells and the cells were washed with ice-cold PBS. Subsequently, cells were lysed in lysis buffer (25 mmol/L Tris–HCl, pH 7.4, 0.5 mmol/L ethyleneglycotetraacetic acid, 25 mmol/L NaCl, 1% Nonidet P-40, 1 mmol/L Na_3VO_4, 10 mmol/L NaF, 0.2 mmol/L leupeptin, 1 mmol/L benzamidine, and 0.1 mmol/L 4-[2-aminoethyl]benzenesulfonyl fluoride hydrochloride) and rocked for 30 minutes at 4°C. Detergent insoluble material was sedimented by centrifugation at 12 000*g* for 10 minutes at 4°C. Protein concentration of the cell lysates was determined by bicinchoninic acid assay (Pierce Chemical Co, Rockford, Ill). For analysis of p38 phosphorylation status and protein levels, cells were starved in DMEM/0.5% BSA for 2 hours, followed by treatment with A304000 (50 μmol/L) or vehicle (DMSO) for 30 minutes before stimulation with insulin (1 μmol/L). At the indicated time points, the cells were rinsed briefly in ice-cold PBS and immediately lysed in 1× SDS-PAGE sample buffer.

2.5. Immunoprecipitation

Insulin receptor substrate 1 was immunoprecipitated from 150 μg of total protein. Lysates were diluted to 0.5 μg/μL in lysis buffer. The lysates were precleared with the addition of protein-A-agarose beads (Invitrogen, Carlsbad, Calif) and rocked at 4 °C for 30 minutes. Beads were sedimented by brief centrifugation at no more than 2500*g* at 4°C. Supernatants were transferred to new tubes and 1 μg of rabbit anti-IRS-1 (Upstate Biotechnology, Charlottesville, Va) was added to each immunoprecipitation. Samples were rocked for 90 minutes at 4°C, before addition of 50 μL of protein-A-agarose beads and incubated for 45 minutes at 4°C with rocking. Subsequently, beads were washed 3 times with lysis buffer and denatured by the addition sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer. Samples were then subjected to immunoblot analysis.

2.6. Immunoblotting

Ten micrograms of total protein was separated on 10% acrylamide SDS-PAGE gels (BioRad, Hercules, Calif) for analysis of GLUT1, GLUT4, phospho-PKB, total PKB, phosphor-p38, total p38, and actin. Insulin receptor substrate 1 immunoprecipitates were separated on 7.5% acrylamide SDS-PAGE gels for analysis of phosphor-tyrosine, IRS-1, and p85 PI 3 kinase. Proteins were transferred to nitrocellulose (Millipore, Billerica, Mass). Membranes were blocked in 4% nonfat dry milk in Tris-buffered saline with 0.5% Tween 20 (TBS-T, Sigma), and primary antibodies diluted in either 4% nonfat dry milk/TBS-T or 0.1% BSA/TBS-T. Antiphosphotyrosine (4G10), IRS-1, and the p85 regulatory subunit of PI 3 kinase (whole antiserum) were from Upstate Biotechnology; GLUT4 was from Chemicon, Temecula, Calif; GLUT1 was from SW Cushman, NIH; phospho-p38 and total p38 were from Cell Signaling Technology, Madison, Wis; actin antibody was from Santa Cruz Biotechnology, Santa Cruz, Calif. Membranes were washed thoroughly and incubated with the appropriate secondary antibody (antibodies conjugated to horseradish peroxidase, Amersham) or Horseradish peroxidase-conjugated protein A (Amersham). After washing, protein bands were visualized using enhanced chemiluminescence (Amersham) and exposure to Hyperfilm ECL x-ray film (Amersham). Densitometric analysis of the bands was performed using a laser densitometer (personal densitometer SI, Amersham) and analyzed by ImageQuant software (Amersham).

2.7. Statistical analysis

Data are presented as means ± SE. Statistical significance was set at $P < .05$. Statistically significant differences were determined by analysis of variance (JMP version 3.1, SAS Institute, Cary, NC). A least significant difference test was used for post hoc analysis.

3. Results

3.1. Inhibition of p38 partially recovered insulin-stimulated glucose uptake

We have previously demonstrated that inhibition of p38 can prevent the loss of GLUT4 protein, but not IRS-1, in a cellular model of insulin resistance [13]. Thus, we wished to determine if an increase in GLUT4 without an improvement in insulin signaling would have a beneficial effect upon glucose uptake. Acute insulin stimulation resulted in approximately 5-fold increase in glucose uptake (Fig. 2). Prior exposure to p38 inhibitor A304000 [12,13] did not significantly alter glucose uptake in response to acute insulin stimulation in insulin-sensitive cells. This is consistent with Somwar et al [11,12], who demonstrated that the inhibitory effects of p38 inhibitors upon glucose uptake are reversible. Exposure to insulin for 20 hours had no effect upon basal glucose uptake, but reduced the insulin response to subsequent insulin stimulation to only a 2.6-fold increase in glucose uptake rate. In response to a subsequent insulin stimulation, A304000-treated cells had a significantly higher glucose uptake rate compared to vehicle-treated cells; however, the fold increase in glucose uptake rate over basal was only 2.1-fold. Similar results were observed with the structurally unrelated p38 inhibitor SB203580 (data not shown).

The effects of A304000 upon glucose uptake were dose responsive, as lower doses of A304000 resulted in proportional changes in glucose uptake (data not shown). The concentration of A304000 used in these experiments (50 μmol/L) was previously demonstrated to be the maximal dose for preventing the loss of GLUT4 levels and capable of inhibiting the insulin-stimulated phosphorylation of p38 [13] and provided the greatest effect upon glucose uptake in the current experiments. As previously demonstrated [12,13], phosphorylation of p38 is rapidly and

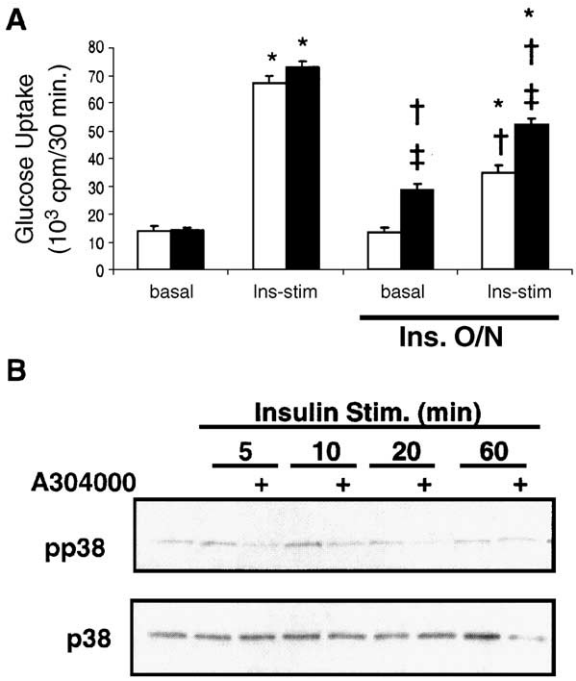

Fig. 2. Inhibition of p38 improves glucose uptake in insulin-resistant 3T3-L1 adipocytes—3T3-L1 adipocytes were treated as described in "Experimental design and methods" section. A, Glucose uptake reported as ^{3}H 2-deoxyglucose incorporation. Data reported as means and SE from at least 6 independent observations. Vehicle-treated cells (DMSO, white bars) and A304000 (50 μmol/L, black bars). B, Whole cell lysates were prepared and subjected to SDS-PAGE, transfer, and immunoblotting for phospho-p38 or total p38 as described in the methods. Asterisk indicates significant difference from basal ($P < .05$); dagger, significant difference between acute and insulin overnight (O/N) ($P < .05$); double dagger, significant difference from vehicle ($P < .05$).

transiently increased by insulin stimulation, which could be prevented by inhibition of p38 with A304000 (Fig. 2B).

3.2. Mechanisms of A304000-mediated improvement in glucose uptake

p38 inhibitors were previously shown to prevent the down-regulation of GLUT4 protein levels, but not the degradation IRS-1 [13]. Thus, it was our hypothesis that the increased insulin-mediated glucose uptake levels observed in A304000-treated adipocytes would be the result of elevated GLUT4 levels and not an improvement in insulin signaling. Acute insulin stimulation increased tyrosine phosphorylation of IRS-1, which was reduced in cells stimulated after a chronic exposure to insulin. As expected, chronic insulin treatment resulted in a decrease in IRS-1 protein levels that also manifested itself in a decreased level of tyrosine phosphorylation and association with the p85 regulatory subunit of PI 3 kinase (Fig. 3A). Treatment with A304000 had no effect upon IRS-1 tyrosine phosphorylation, IRS-1 protein levels, or association with p85 in response to acute insulin stimulation of insulin-sensitive cells. Likewise, A304000 did not prevent the decrease in IRS-1 protein levels and associated signaling in cells made insulin resistant by chronic treatment with insulin.

PKB is a downstream kinase of IRS-1 and PI 3 kinase and is a critical step in insulin-mediated glucose uptake [15]. Phosphorylation of PKB was increased by acute insulin stimulation (Fig. 3B), whereas chronic treatment with insulin substantially reduced PKB phosphorylation in response to subsequent acute insulin stimulation, consistent with decreased insulin signaling through IRS-1. A304000 had no effect upon PKB phosphorylation in response to acute insulin stimulation, nor did the inhibitor affect the down-regulation of PKB phosphorylation after chronic insulin treatment.

As expected, protein levels of the insulin-responsive glucose transporter GLUT4 were decreased by chronic exposure to insulin (Fig. 3C). Consistent with our previous observations, inhibition of p38 during the development of insulin resistance prevented the decrease in GLUT4 protein. Protein levels of GLUT1, the basal glucose transporter, were not altered by chronic insulin in vehicle-treated cells. However, treatment of the cells with A304000 increased GLUT1 protein levels, regardless of exposure to chronic insulin.

3.3. Rapamycin and A304000 exert additive effects upon glucose uptake in insulin-resistant cells

Treatment of adipocytes with rapamycin can prevent the loss of IRS-1, leading to a partial recovery of insulin

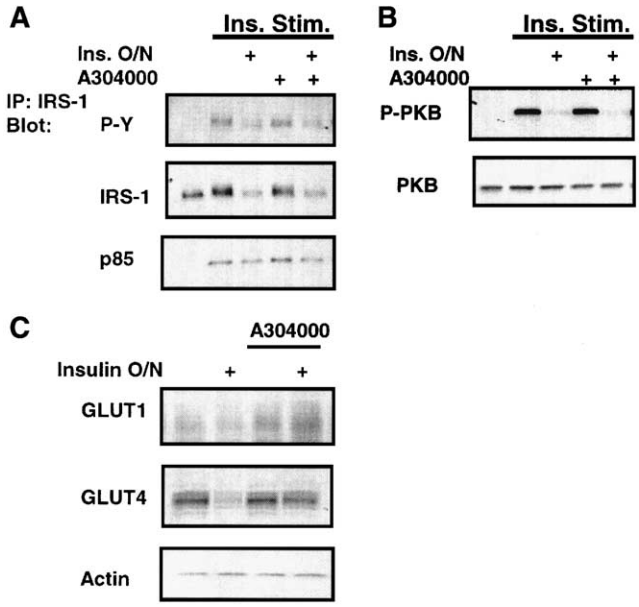

Fig. 3. Effects of A304000 upon insulin signaling and glucose transporter protein levels. 3T3-L1 adipocytes were treated as described in "Experimental design and methods" section, except that cell lysates were prepared from cells treated in parallel to cells undergoing glucose uptake measurements and subjected to 5-minute acute insulin stimulation. Insulin receptor substrate 1 was immunoprecipitated from equivalent amounts of cellular protein and subjected to SDS-PAGE and western blotting for phosphotyrosine, IRS-1, or p85 (A). Whole cell lysates were prepared, subjected to SDS-PAGE and western blotting for phospho-PKB and total PKB (B), or GLUT1, GLUT4, and actin (C). Images are representative of at least 3 independent replications. Ins Stim indicates insulin stimulation.

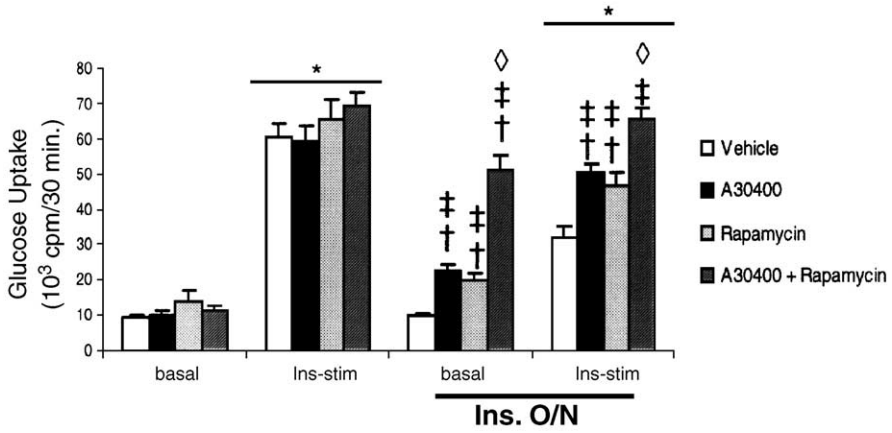

Fig. 4. Inhibition of mTOR and p38 has additive effects upon glucose uptake—3T3-L1 adipocytes were treated as described in "Experimental design and methods" section. Glucose uptake was reported as ^{3}H 2-deoxyglucose incorporation. Data are reported as means and SE from at least 6 independent observations. Vehicle-treated cells (DMSO, white bars), A304000-treated cells (50 μmol/L, black bars), rapamycin (200 nmol/L, light gray bars), or A304000 and rapamycin (dark gray bars). Asterisk indicates significant difference from basal ($P < .05$); dagger, significant difference between acute and chronic insulin ($P < .05$); double dagger, significant difference from vehicle ($P < .05$), diamond, significant difference from A304000 alone or rapamycin alone ($P < .05$).

signaling to PKB and insulin-stimulated glucose uptake, without improved GLUT4 protein levels [16]. Thus, p38 and mTOR act upon different pathways during the development of insulin resistance; therefore, we hypothesized that inhibition with both A304000 and rapamycin would be additive. Treatment with A304000 alone, rapamycin alone, or A304000 and rapamycin had no effect upon glucose uptake in response to an acute insulin stimulation (Fig. 4). In response to chronic insulin stimulation, both A304000 and rapamycin increased basal glucose uptake, with a further increase observed when the cells were treated with both inhibitors. Chronic insulin decreased glucose uptake in response to a subsequent acute insulin stimulation in vehicle-treated cells, and insulin-stimulated glucose uptake was partially restored by treatment with A304000 or rapamycin (Fig. 4). When these inhibitors were used in combination, insulin-stimulated glucose uptake could be increased to the levels of vehicle-treated cells in response to acute insulin stimulation. However, this effect was not mathematically additive.

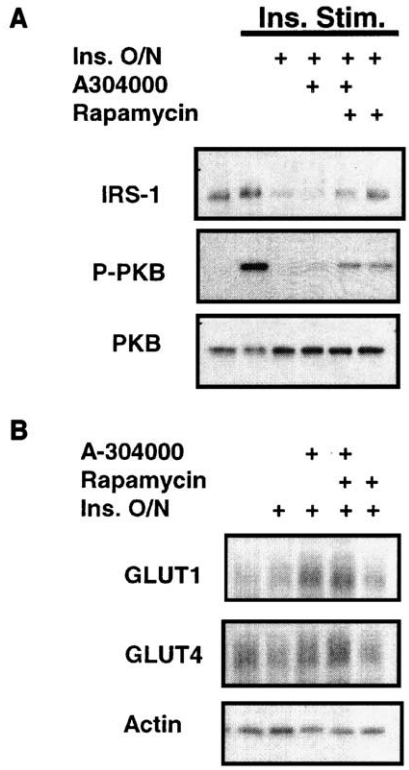

Fig. 5. Inhibition of mTOR or p38 have differential effects upon insulin signaling and glucose transporter protein levels—3T3-L1 adipocytes were treated as described in "Experimental design and methods" section, except that cell lysates were prepared from cells treated in parallel to cells undergoing glucose uptake measurements, and subjected to 5-minute acute insulin stimulation. Lysates were prepared as described in "Experimental design and methods" section and subjected to SDS-PAGE and western blotting for IRS-1, phosphor-PKB, total PKB (A), GLUT1, GLUT4, or actin (B). Images are representative of at least 3 independent observations.

To confirm that the mechanisms underlying the effects rapamycin and A304000 upon glucose uptake did not overlap, we demonstrated that IRS-1 protein levels and PKB phosphorylation were improved with rapamycin treatment, but not by A304000 (Fig. 5A). Furthermore, results from treatment with both inhibitors were not additive. Likewise, A304000 treatment increased protein levels of both GLUT4 and GLUT1, whereas rapamycin had no effect (Fig. 5B). Treatment with both rapamycin and A304000 did not have an additive effect upon GLUT4 or GLUT1 protein levels.

4. Discussion

We have previously demonstrated that p38 phosphorylation is increased in adipocytes from type 2 diabetics, a condition associated with decreased GLUT4 and IRS-1 protein levels [13]. Furthermore, inhibition of p38 could prevent the loss of GLUT4 in a cellular model of insulin resistance. However, improvements in GLUT4 were not

accompanied by an improvement in IRS-1 protein levels, indicating that p38 inhibitors would not improve defects in insulin signaling. Thus, it was unclear whether p38 inhibition would have a beneficial effect upon insulin-stimulated glucose uptake. We examined the functional aspects of p38 inhibition during the development of insulin resistance to resolve this issue. Inhibition of p38 increased basal glucose uptake as well as insulin-stimulated glucose uptake, likely through increased expression of GLUT1 and GLUT4 protein levels. However, the fold change in glucose uptake rate in response to insulin was not improved, consistent with the observation that inhibition of p38 did not improve insulin resistance at the level of insulin signaling. This is in contrast to treatment with rapamycin, an inhibitor of mTOR, which partially recovered insulin signaling and glucose uptake but not GLUT4 protein levels. Thus, we anticipated that inhibition of both p38 and mTOR would result in an additive increase in glucose uptake. As expected, combined use of the inhibitors resulted in improved glucose uptake, further confirming that p38 and mTOR are involved with distinct mechanisms in the development of insulin resistance.

p38 has a complex role in the regulation of glucose uptake. Recent work has demonstrated that p38 is necessary for insulin-stimulated glucose uptake [11,12]. Inhibition of p38 can prevent insulin-stimulated glucose uptake, but not GLUT4 translocation, suggesting that p38 may regulate a yet to be discovered activation step [11,12]. Thus, in acute situations, p38 is beneficial to GLUT4-mediated glucose uptake. However, p38 also contributes to the development of insulin resistance, as inhibition of p38 improved GLUT4 protein levels and insulin-stimulated glucose uptake. The detrimental effects of p38 activation are further supported by the observation that constitutive activation of the p38 pathway decreased GLUT4 messenger RNA and protein levels as well as insulin-stimulated glucose uptake [14]. Taken together, these results suggest that p38 has acute effects, potentially at the cell membrane to up-regulate insulin-stimulated glucose uptake, and chronic effects involving the down-regulation of GLUT4 protein levels, potentially by targeting gene expression [14]. Distinct cellular compartmentalization of p38 signaling is an intriguing explanation for these divergent roles of p38 in glucose uptake.

The mechanisms by which p38 regulates the protein levels of GLUT1 and GLUT4 are unknown. 3T3-L1 adipocytes have a basal level of p38 phosphorylation and activity that can be transiently increased by insulin stimulation [9-13]. Inhibition of p38 alone did not increase GLUT4 protein levels, but did increase GLUT1, suggesting that basal activity of p38 is important for the suppression of GLUT1 expression, but not sufficient for down-regulation of GLUT4. Because p38 may play a role in regulating GLUT4 activity [11,12], inhibition of p38 may inhibit glucose uptake and drive GLUT1 expression. Incubation of 3T3-L1 adipocytes in low glucose media can increase GLUT1 expression [17], suggesting that diminished glucose uptake may be capable of inducing GLUT1.

Inhibition of p38 resulted in increased basal glucose uptake, which was likely the source of the improved insulin-stimulated glucose uptake, as p38 inhibition did not improve insulin sensitivity. Indeed, the fold increase of glucose uptake by insulin was slightly decreased from 2.6-fold to 2.1-fold. This may represent a detrimental effect of increased GLUT1-mediated glucose uptake, because transgenic overexpression of GLUT1 in skeletal muscle promotes hexosamine biosynthesis and potentially insulin resistance [18].

Up-regulation of GLUT4 is a hypothetical therapeutic option to improve glucose tolerance in insulin-resistant and diabetic conditions. Several experiments have used transgenic overexpression of GLUT4 and/or GLUT1 in mouse models of insulin resistance and diabetes [19-21]. Although overexpression of glucose transporters could improve glucose tolerance, it was not capable of preventing insulin resistance or diabetes [19-21]. This is consistent with our results that increasing GLUT4 by p38 inhibition can improve glucose uptake but does not improve insulin resistance, thus, additive effects can be observed when insulin sensitivity is improved by additional rapamycin treatment.

In conclusion, inhibition of p38 can increase basal glucose uptake in conjunction with increased protein levels of GLUT1 and GLUT4. This resulted in improved insulin-stimulated glucose uptake in the absence of an improvement in insulin sensitivity. Although increased expression of GLUT4 can improve glucose tolerance in obese animals, treatment with p38 inhibitors may not be an optimal treatment strategy because of their failure to improve insulin sensitivity, and the complex role p38 may play in regulating glucose uptake and other cellular processes.

Acknowledgments

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