MKK6/3 and p38 MAPK Pathway Activation Is Not Necessary for Insulin-induced Glucose Uptake but Regulates Glucose Transporter Expression*

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p38 mitogen-activated protein kinase (MAPK), which is situated downstream of MKK kinase (MKK) 6 and MKK3, is activated by mitogenic or stress-inducing stimuli, as well as by insulin. To clarify the role of the MKK6/3-p38 MAPK pathway in the regulation of glucose transport, dominant negative p38 MAPK and MKK6 mutants and constitutively active MKK6 and MKK3 mutants were overexpressed in 3T3-L1 adipocytes and L6 myotubes using an adenovirus-mediated transfection procedure. Constitutively active MKK6/3 mutants up-regulated GLUT1 expression and down-regulated GLUT4 expression, thereby significantly increasing basal glucose transport but diminishing transport induced by insulin. Similar effects were elicited by chronic (24 h) exposure to tumor necrosis factor α, interleukin-1β, or 200 mM sorbitol, all activate the MKK6/3-p38 MAPK pathway. SB203580, a specific p38 MAPK inhibitor, attenuated these effects, further confirming that both MKK6 and MKK3 act via p38 MAPK, whereas they had no effect on the increase in glucose transport induced by a constitutively active MAPK kinase 1 (MEK1) mutant or by myristoylated Akt. In addition, suppression of p38 MAPK activation by overexpression of a dominant negative p38 MAPK or MKK6 mutant did not diminish insulin-induced glucose uptake by 3T3-L1 adipocytes. It is thus apparent that activation of p38 MAPK is not essential for insulin-induced increases in glucose uptake. Rather, p38 MAPK activation leads to a marked down-regulation of insulin-induced glucose uptake via GLUT4, which may underlie cellular stress-induced insulin resistance caused by tumor necrosis factor α and other factors.

Virtually all mammalian cells utilize glucose as a major energy source and thus possess facilitative glucose transporters on their cell surfaces. The facilitative glucose transporter gene family encodes at least six isoforms (GLUT1–6) with varying tissue distributions, subcellular localizations, and kinetic properties for glucose uptake (1, 2). Among them, GLUT4, in which expression is strictly limited to muscle and fat cells, resides in an intracellular compartment under basal conditions and moves to the cell surface in response to stimulation by insulin and other factors. In contrast, the ubiquitously expressed GLUT1 is mainly located on the cell surface, irrespective of stimulation, and is thus considered to be mainly involved in the maintenance of basal glucose uptake into the cells.

The mechanisms whereby signal-transducing molecules regulate the activity of glucose transporters have been extensively studied. At present, it is clear that activation of phosphatidylinositol 3-kinase 3-kinase is essential for insulin-induced GLUT4 translocation to the plasma membrane and increased glucose uptake (3–6), although the mediators downstream of phosphatidylinositol 3-kinase remain controversial (7–9). In addition, activation of classical mitogen-activated protein kinase (MAPK) (also termed extracellular signal-regulated kinase (ERK)), which plays a central role in cellular transformation, reportedly up-regulates GLUT1 expression, thereby augmenting glucose transport (10, 11).

Recent studies have revealed the existence of at least three independent MAPK pathways (12–14). Besides ERK, two novel MAPKs have been identified and designated p38 MAPK (15, 16) and stress-activated protein kinase (also known as c-Jun N-terminal kinase (JNK)) (8, 17). Among the mitogenic factors and stress-inducing stimuli that mediate activation of these enzymes, insulin is reported to activate p38 MAPK (18, 19). Interestingly, SB203580, a specific inhibitor of p38 MAPK, inhibits insulin-stimulated increases in glucose transport in both 3T3-L1 adipocytes and L6 myotubes without affecting insulin-stimulated GLUT4 translocation to the cell surface (20). It has therefore been hypothesized that p38 MAPK activation is involved in an insulin-induced enhancement of intrinsic GLUT4 activity on the cell surface. In addition, p38 MAPK is also activated in response to tumor necrosis factor (TNF) α, interleukin 1 (IL-1), and hyperosmotic shock (21–23), reportedly leading to insulin resistance (24–27).

In that context, our study was undertaken to clarify the role of the p38 MAPK pathway in the regulation of glucose trans-
FIG. 1. Overexpression of MAPK family mutants. 3T3-L1 adipocytes were infected with recombinant adenoviruses containing LacZ (control) or the indicated mutants as follows. A, LASDSE-MEK1; B, AA-MKK6, EE-MKK6, EE-MKK3, and p38 MAPK-AP; and C, DED-MKK7. Equal amounts of protein isolated from the cells were subjected to SDS-PAGE and then immunoblotted using specific antibodies as probes.

To modulate p38 MAPK activity, dominant negative p38 MAPK and MAPK kinase (MKK) mutants as well as constitutively active MKK mutants were overexpressed in 3T3-L1 adipocytes and L6 myotubes using an adenovirus-mediated transfection system. In addition, the effects induced by TNFα, IL-1β, and 200 mM sorbitol were examined in 3T3-L1 adipocytes. We show that MKK6- and MKK3-mediated activation of p38 MAPK significantly alters expression of GLUT1 and GLUT4, increasing basal glucose transport and reducing insulin responsiveness.

EXPERIMENTAL PROCEDURES

Materials—3-Isobutyl-1-methylxanthine and 2-deoxy-D-glucose were purchased from Wako Bioproducts. Enhanced chemiluminescence (ECL) detection system was from Amersham Pharmacia Biotech. TNFα and IL-1β were from Genzyme Corp. Inhibitors Wortmannin and SB203580 were from Sigma and Calbiochem, respectively. [γ-32P]UTP was from ICN. All other reagents from commercial sources were of analytical grade.

Antibodies—Anti-MAPK kinase 1 (MEK1), anti-MKK3, anti-MKK6, and anti-MKK7 antibodies were purchased from Santa Cruz Biotechnology. Anti-p38 MAPK and anti-phospho-p38 MAPK (Thr180/Tyr182) were from New England Biolabs. Anti-GLUT1 and anti-GLUT4 antibodies were raised against the C-terminal region of GLUT1 (28) and GLUT4 (29), respectively.

Cell Culture—3T3-L1 fibroblasts were initially maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (Life Technologies, Inc.) under an atmosphere of 90% air, 5% CO2 at 37 °C. Differentiation was induced 2 days after the cells reached confluence by replacing their normal culture medium with DMEM supplemented with 5 mM 3-isobutyl-1-methylxanthine, 4 μg/ml dexamethasone (Sigma), and 10% fetal bovine serum (Life Technologies, Inc.) for 48 h. Thereafter, the cells were incubated for an additional 4–10 days in DMEM supplemented with 10% fetal bovine serum; the medium was changed every other day. With this protocol, more than 90% of the cells expressed the adipocyte phenotype (3).

L6 myoblasts were initially grown in DMEM supplemented with 10% fetal calf serum under an atmosphere of 95% air, 5% CO2 at 37 °C, after which they were seeded onto 10-cm plastic culture dishes at a density of 3,000 cells/cm2. To promote their fusion into myotubes, they were rendered quiescent by incubation for 10 days in DMEM containing 2% serum. Myotube formation was assessed according to the percentage of nuclei present in multinucleated myotubes. With this protocol, 80–90% of myoblasts were fused into myotubes.

Construction of MAPK Mutants and Myristoylated Akt—Plasmids containing the cDNAs encoding constitutively active MEK1 (LASDSE-MEK1), MKK3 (EE-MKK3), MKK6 (EE-MKK6), and MKK7 (DED-MKK7) mutants and dominant negative MKK6 (AA-MKK6) and p38 MAPK (p38 MAPK-AP) mutants were prepared as follows. LASDSE-MEK1 was constructed by substituting serine 218 with aspartic acid, serine 222 with glutamic acid, and two critical leucines (Leu17 and Leu24) in the nuclear export signal sequence of Xenopus MAPK with alanines (30). EE-MKK3 was constructed by replacing serine 189 and threonine 193 with glutamic acids (31). EE-MKK6 and AA-MKK6 was constructed by substituting serine 207 and threonine 111 with glutamic acid and alanine, respectively (31). p38 MAPK-AP was constructed by substituting threonine 180 and threonine 182 in the TGY motif with alanine and phenylalanine, respectively (31). DED-MKK7 was constructed by substituting serine 287 with aspartic acid, threonine 291 with glutamic acid, and serine 293 with aspartic acid. Myristoylated Akt (Myr-Akt), which contains an src myristoylation signal sequence, was described previously (32).

Gene Transduction—To obtain recombinant adenoviruses, the expression cosmid cassette pAdexCAwt was ligated with the cDNA encoding LacZ from Escherichia coli or one of the aforementioned mutants, respectively, after which homologous recombination between the recombinant cosmid cassette and its parental virus genome was carried out as described previously (33). Infection of 3T3-L1 (5, 34) and L6 cells (35) with the indicated adenoviruses was carried out as described previously. Assessing triglyceride content of the infected cells confirmed that overexpression of the MAPK family mutants did not affect differentiation of 3T3-L1 cells into adipocytes (data not shown).

Immunoblotting—3T3-L1 adipocytes in a 12-well tissue culture dish were lysed, boiled in Laemmli buffer containing 10 mM dithiothreitol, and subjected to SDS-polyacrylamide gel electrophoresis (PAGE). Immunoblotting was performed using an ECL system according to the manufacturer’s instructions.

p38 MAPK Assay—Adipocytes were serum-deprived for 3 h before being exposed to 100 nM insulin for 5 min. p38 MAPK activity was assayed using a specific kit (New England Biolabs) according to the manufacturer’s instructions. Briefly, the cells were lysed, immunoprecipitated with immobilized anti-phospho-p38 MAPK (Thr180/Tyr182) antibody, and immunoblotted with anti-phospho-ATF2 (Thr71) antibody, a natural substrate of p38 MAPK.

Glucose Transport Assay—3T3-L1 adipocytes and L6 myotubes in 24-well culture dishes were serum-starved for 3 h in DMEM containing 0.2% bovine serum albumin, and then they were incubated for 45 min in glucose-free Krebs-Ringer phosphate buffer (in mM: 137 NaCl, 4.7 KCl, 10 sodium phosphate (pH 7.4), 0.5 MgCl2, and 1 CaCl2) (3). Basal and stimulated uptake of 2-deoxy-D-[3H]glucose was then measured as described previously (29). Experiments were carried out in the presence and absence of glucose transport inhibitors.

Subcellular Fractionation—Subcellular membranes from 3T3-L1 adipocytes were carried out essentially as described previously (36). Aliquots of subcellular membrane fractions containing equal amounts of protein were subjected to SDS-PAGE, followed by immunoblotting using anti-GLUT1 and anti-GLUT4 antisera and an ECL detection system.

RNA Extraction—Total cell RNA was isolated from 3T3-L1 adipocytes using an Ison IA RNA isolation kit (Nippon Gene, Tokyo, Japan). RNA concentrations were estimated based on absorbance at 260 nm, and 10 μg of RNA from each sample were used for the RNase protection assay described below.

Preparation of Riboprobes—Riboprobes were synthesized as de-
**RESULTS**

**Overexpression of MAPK Family Mutants in 3T3-L1 Adipocytes—**LASDSE-MEK1, p38 MAPK- AF, AA-MKK6, EE-MKK6, EE-MKK3, and DED-MKK7 were overexpressed in 3T3-L1 adipocytes using an adenovirus transfection system (Fig. 1). Their respective levels of expression were 5-, 11-, 10-, 15-, 8-, and 8-fold greater than that in control cells overexpressing LacZ. Similar results were obtained with L6 myotubes (data not shown).

**Effects of p38 MAPK and MKK6 Mutants on p38 MAPK Activity in 3T3-L1 Adipocytes in the Presence and Absence of Insulin—**p38 MAPK, which is activated by insulin-mediated dual phosphorylation on threonine 180 and tyrosine 182 in the TGY motif, phosphorylates a number of downstream targets, including ATF2. We overexpressed p38 MAPK-AF, AA-MKK6, EE-MKK6, and EE-MKK3 in 3T3-L1 adipocytes, after which p38 MAPK activities were measured by immunoblotting anti-phospho-p38 MAPK (Thr180/Tyr182) immunoprecipitates with anti-phospho-ATF2 (Thr71) antibody (Fig. 2). As shown previously, insulin did indeed activate p38 MAPK in control 3T3-L1 adipocytes overexpressing LacZ. By contrast, overexpression of EE-MKK6 led to marked activation of p38 MAPK irrespective of insulin stimulation, whereas overexpression of p38 MAPK-AF or AA-MKK6 strongly inhibited insulin-induced activation of p38 MAPK. EE-MKK3 also activated p38 MAPK; however, the magnitude of the enhancement was smaller than seen with EE-MKK6 (data not shown).

**Effect of Dominant Negative p38 MAPK and MKK6 Mutants on Glucose Transport in 3T3-L1 Adipocytes—**Fig. 3A shows the basal and insulin-stimulated 2-deoxy[3H]glucose uptake into 3T3-L1 adipocytes overexpressing LacZ or dominant negative p38 MAPK (p38-AF) and MKK6 (AA-MKK6) mutants. In each case, insulin markedly enhanced glucose uptake. Moreover, overexpression of either p38-AF or AA-MKK6 significantly enhanced the insulin-induced increases in glucose uptake, with the latter having the greater degree. Based on these results, it seems clear that p38 MAPK activation is not essential for insulin-induced increases in glucose uptake.

On the other hand, overexpression of a constitutively active form of MKK6 (EE-MKK6) led to an ∼30-fold increase in glucose uptake by 3T3-L1 adipocytes, even in the absence of insulin, and insulin stimulation further increased glucose uptake by ∼50% (Fig. 3B). Pretreatment for 15 min with 100 nM wortmannin, a phosphatidylinositol 3-kinase inhibitor, abolished the insulin-induced increase in glucose uptake by cells overexpressing either LacZ or EE-MKK6, although wortmannin had no effect on the increase in glucose uptake induced by EE-MKK6.

**Comparison of the Effects of Constitutively Active MKK Mutants on Glucose Transport in 3T3-L1 Adipocytes and L6 Myotubes—**As mentioned in the Introduction, there exist at least three MAPK pathways. To examine the roles of the respective MKK isoforms involved in these pathways, the effects of overexpressing constitutively active forms of MKK3 (EE-MKK3), MKK6 (EE-MKK6), MEK1 (LASDSE-MEK1), and MKK7 (DED-MKK7) in 3T3-L1 adipocytes and L6 myotubes were compared. Maximal overexpression of EE-MKK3, EE-MKK6, and LASDSE-MEK1 induced ∼3-, 30-, and 15-fold, respectively, increases in glucose uptake by 3T3-L1 adipocytes, whereas DED-MKK7 had no effect on glucose transport (Fig. 4A).

As reported previously, a membrane-targeted, constitutively active form of Akt (myr-Akt) also increased glucose transport in both 3T3-L1 adipocytes and L6 myotubes (Figs. 4A and B).

**Effect of Overexpressing EE-MKK6 or LASDSE-MEK1 on the Subcellular Distribution of Glucose Transporter in 3T3-L1 Adipocytes—**To understand better the mechanism underlying the increase in glucose transport induced by MKK6 and MEK1, subcellular fractionation of 3T3-L1 adipocytes was performed, and the GLUT1 and GLUT4 contents of the plasma membrane (PM) and low density microsome (LDM) fractions were analyzed. We found that EE-MKK6 and LASDSE-MEK1 markedly
increased levels of GLUT1 protein in both the PM and LDM fractions (Figs. 5, A and B), whereas levels of GLUT4 protein were markedly diminished (Fig. 5, C and D).

**Effect of Overexpressing EE-MKK6 or LASDSE-MEK1 on Expression of Glucose Transporter mRNA in 3T3-L1 Adipocytes—**By using an RNase protection assay, we found that, as compared with control (LacZ), expression of GLUT1 mRNA was significantly increased, whereas that of GLUT4 mRNA was significantly diminished in 3T3-L1 adipocytes overexpressing either EE-MKK6 or LASDSE-MEK1 (Fig. 6).

**Time-dependent Effect of SB203580 on Stimulated Glucose Transport in 3T3-L1 Adipocytes Overexpressing EE-MKK6—**To confirm whether or not MKK6-induced changes in glucose transport and GLUT1/4 expression were mediated via p38 MAPK, the effects of SB203580, a specific p38 MAPK inhibitor, were examined. As shown in Fig. 7, SB203580 time-dependently inhibited the increase in glucose uptake induced by overexpression of EE-MKK6 in 3T3-L1 adipocytes; an almost complete blockade was achieved when cells were preincubated with SB203580 for 48 h.

By contrast, preincubation with SB203580 had no significant effect on the increase in glucose uptake induced by overexpression of myr-Akt.

**Effects of SB203580 on Glucose Transport and Expression of GLUT1/4 in 3T3-L1 Adipocytes Overexpressing Constitutively Active MKK Mutants—**Preincubation for 24 h with 10 μM SB203580 significantly inhibited the increase in glucose transport normally induced by overexpression of EE-MKK6 and EE-MKK3 in 3T3-L1 adipocytes but not that induced by LASDSE-MEK1 (Fig. 8). Moreover, preincubation with SB203580 restored expression of both GLUT1 and GLUT4 protein to control levels in 3T3-L1 adipocytes overexpressing EE-MKK3 or EE-MKK6 (Fig. 9). By contrast, the effects of overexpressing LASDSE-MEK1 on expression of GLUT1 and GLUT4 protein were unaffected by SB203580.

**Effects of TNFα, IL-1β, and Hyperosmolarity on p38 MAPK Phosphorylation, Glucose Transport, and GLUT1 Expression in 3T3-L1 Adipocytes—**As shown in Fig. 10, briefly (30 min) exposing 3T3-L1 adipocytes to TNFα, IL-1β, or 200 mM sorbitol up-regulated phosphorylation of p38 MAPK. Consistent with these findings, more prolonged (24 h) exposures to these stimuli significantly elevated glucose transport in 3T3-L1 adipocytes (Fig. 11A, striped bars). Similar results were obtained with cells overexpressing EE-MKK6 and EE-MKK3 (Figs. 10 and 11A, solid bars). In addition, incubating cells for 24 h with TNFα, IL-1β, or 200 mM sorbitol up-regulated GLUT1 expression (Fig. 11B, striped bars) and down-regulated GLUT4 expression (data not shown) in a manner similar to that seen with overexpression of EE-MKK6 and EE-MKK3 (Fig. 11B, solid bars).
Glucose transport is known to be affected by cell differentiation and oncogenic transformation, as well as by various hormones and growth factors. Such modulation has often been attributed to altered expression of one or more glucose transporter isoforms (i.e., GLUT1–6) (1, 2) or to modulation of glucose transporters’ (particularly GLUT4) capacity to translocate to the plasma membrane (38–40). The current study was undertaken to understand better the role of MKK6/3-p38 MAPK activation on the regulation of glucose transport and glucose transporter expression.

Activation of p38 MAPK Is Not Essential for Insulin-induced Increases in Glucose Uptake—It was recently reported that treatment with SB203580, a specific p38 MAPK antagonist, inhibited insulin-induced glucose uptake by both 3T3-L1 adipocytes and L6 myotubes, without inhibiting translocation of GLUT1 or GLUT4 (20). Consequently, it was hypothesized that activation of p38 MAPK plays a key role in the insulin-induced enhancement of intrinsic GLUT4 activity on the cell surface. On the other hand, p38 MAPK is also known to be activated by stressful stimuli, such as TNFα, IL-1, and hyperosmotic shock (21–23), which appear to induce insulin resistance (41–43). To clarify whether or not activation of p38 MAPK is indeed essential for insulin-induced increases in glucose transport, we overexpressed dominant negative p38 MAPK and MKK6 mutants, which markedly suppressed endogenous p38 MAPK activity but significantly enhanced insulin-induced glucose uptake by 3T3-L1 adipocytes. We also observed that 10 μM SB203580 almost completely blocked insulin-induced activation of p38 MAPK but that the insulin-induced increase in glucose uptake was reduced by only 30% (data not shown). A higher concentration of SB203580 (100 μM) inhibited the insulin-induced increase in glucose uptake by more than 90%; however, at this concentration, SB203580 also significantly suppressed the activity of Akt kinase (data not shown). It therefore appears that the inhibition of insulin-induced increases in glucose uptake by SB203580 was not mediated by an effect on p38 MAPK activity but by an effect on some other molecule(s), perhaps Akt or GLUT4 itself. Furthermore, it appears unlikely that activation of p38 MAPK plays a significant role in the regulation of the insulin-induced increases in glucose transport mediated by GLUT4.

Activation of p38 MAPK Leads to Up-regulation of GLUT1 and Down-regulation of GLUT4—We found that the overexpression of constitutively active MKK6 or MKK3 mutants markedly increased expression of GLUT1 protein in both 3T3-L1 adipocytes and L6 myotubes, whereas expression of...
GLUT4 was diminished. These effects were reflected by a significant increase in basal glucose transport and diminished insulin-induced glucose transport, respectively. That SB203580 inhibited MKK6/3-induced alterations in glucose uptake and expression of GLUT1/4 makes it highly likely that p38 MAPK plays a central role in the regulation of glucose metabolism.

Most interesting, overexpression of constitutively active MEK1, which induces chronic activation of ERK, yielded the same result as did overexpression of constitutively active MKK6 and MKK3. Previously, up-regulation of GLUT1 by oncogenes fps, src, and ras or by 12-O-tetradecanoylphorbol-13-acetate was shown to underlie the increased glucose transport related to cellular transformation and/or proliferation (10, 11). The GLUT1 gene contains two enhancer elements as follows: one in the 5′ region contains one serum-response element, two 12-O-tetradecanoylphorbol-13-acetate-response elements (TREs), one cyclic AMP-response element, and three GC boxes; and the other, located in the second intron, contains one cyclic AMP-response element and two TREs (44). Both of these enhancers are involved in the augmented GLUT1 gene transcription induced by ras and v-src, by serum and by platelet-derived growth factor (11, 44, 45). Since transcription activator protein-1 is a target of ERK1/2 (46–48), the association of activator protein-1 with TRE may contribute to the activation of the MEK1-ERK pathway by acting at the 5′ region enhancer(s) of the GLUT1 gene to up-regulate its expression and thus increase glucose transport. Indeed, this phenomenon may be central to the cellular transformation and/or proliferation mediated by activated MEK1-ERK pathway.

MKK6/3 and MKK7 activate p38 MAPK and JNK, respectively (31, 49–52). In 3T3-L1 adipocytes, activation of JNK appears to induce increased synthesis of glycogen (18), and in the present study, we found that MKK6/3, but not MKK7, up-regulated expression of GLUT1 mRNA. Thus, the regulation of GLUT1 gene transcription may not be mediated entirely by activator protein-1 but by a more complex mechanism involving other factors.

On the other hand, the GLUT4 gene, which contains 11 exons and 10 introns, possesses a promoter region containing a weak “TATA” box sequence homology, a CCAAT box to which CCAAT/enhancer binding protein likely binds (53), four potential binding sites for nuclear transcription factor Sp1 (54), a skeletal muscle-specific activation domain (55). Together, these domains play a crucial role in the up-regulation of GLUT4 expression during adipose and muscle cell differentiation. In that respect, we found no significant alteration in cellular triacylglyceride content in 3T3-L1 adipocytes overexpressing the various MAPK family mutants studied; thus the down-regulation of GLUT4 expression in 3T3-L1 adipocytes overexpressing constitutively active forms of MKK6/3 and MEK1 cannot be attributed to the dedifferentiation from adipocytes to fibroblasts.

In many cases, expression of GLUT1 and GLUT4 are regulated oppositely, but whether changes in the expression of one affect the expression of the other remains unknown. If such interdependence exists, down-regulation of GLUT4 might be induced by the elevated intracellular glucose concentration mediated by up-regulation of GLUT1. However, there is reportedly no significant alteration in the GLUT4 expression in the skeletal muscle of transgenic mice overexpressing GLUT1 (56). We therefore suggest that expression of GLUT1 and GLUT4 is regulated independent, although further study will be necessary to resolve this issue definitively.

Involvement of p38 MAPK Activation in the Insulin Resistance Induced by TNFα, IL-1β, and Hyperosmolarity—Several studies have shown that chronically exposing adipocytes to TNFα or oxidant stress markedly decreases their GLUT4 content as a result of decreased GLUT4 gene transcription and a reduced half-life of its mRNA (26, 42, 57, 58). Conversely, chronic exposure to TNFα increases expression of GLUT1 (27, 58, 59), although a contradictory result was obtained in one study (60). In the present study, we found that prolonged (24 h) exposure to TNFα, IL-1β, or hyperosmolarity increased glucose transport, increased GLUT1 expression, and decreased GLUT4 expression in a manner similar to that seen in cells overexpressing constitutively active MKK6. These phenomena were all attenuated by SB203580, implicating p38 MAPK in their occurrence.

Exposure to TNFα, IL-1β, and hyperosmolarity is also known to lead to insulin resistance (24–27). Consistent with the aforementioned findings, down-regulation of GLUT4 and up-regulation of GLUT1 are considered to be components of the mechanism underlying such insulin resistance, although reduced expression of insulin receptor substrate-1 (61) and inhibition of insulin-induced tyrosine phosphorylation of insulin receptor may also be involved in TNFα-induced insulin resistance (41, 62).

In summary, activation of the MKK6/3-p38 MAPK pathway markedly enhances glucose transport by up-regulating GLUT1 expression, irrespective of the stimulus. This effect may help cells meet the increased energetic demands incurred when they are under stress, perhaps determining whether cells survive or succumb to apoptosis, a process in which the MKK6/3-p38 MAPK pathway also plays a role. In addition, although further study will be required to clarify whether the MKK6/3-p38 MAPK pathway is indeed activated under conditions associated with insulin resistance (e.g., obesity), we anticipate that p38 MAPK activation will prove to be a key component of stress-induced insulin resistance in fat and muscle cells.

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