Insulin Signal Transduction Pathways and Insulin-induced Gene Expression*

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Insulin regulates metabolic activity, gene transcription, and cell growth by modulating the activity of several intracellular signaling pathways. Insulin activation of one mitogen-activated protein kinase cascade, the MEK/ERK kinase cascade, is well described. However, the effect of insulin on the parallel p38 pathway is less well understood. The present work examines the effect of inhibiting the p38 signaling pathway by use of specific inhibitors, either alone or in combination with insulin, on the activation of ERK1/2 and on the regulation of gene transcription in rat hepatoma cells. Activation of ERK1/2 was induced by insulin and was dependent on the activation of MEK1, the kinase upstream of ERK in this pathway. Treatment of cells with p38 inhibitors also induced ERK1/2 activation/phosphorylation. The addition of p38 inhibitors followed by insulin addition resulted in a greater than additive activation of ERK1/2. The two genes studied, c-Fos and Pip92, are immediate-early genes that are dependent on the ERK1/2 pathway for insulin-regulated induction because the insulin effect was inhibited by pretreatment with a MEK1 inhibitor. The addition of p38 inhibitors induced transcription of both genes in a dose-dependent manner, and insulin stimulation of both genes was enhanced by prior treatment with p38 inhibitors. The ability of the p38 inhibitors to induce ERK1/2 and gene transcription, both alone and in combination with insulin, was abolished by prior inhibition of MEK1. These data suggest possible cross-talk between the p38 and ERK1/2 signaling pathways and a potential role of p38 in insulin signaling.

Insulin is a primary regulator of energy metabolism, is essential for normal growth and development, and can directly or indirectly stimulate DNA synthesis (1). Upon binding of insulin, the insulin receptor becomes activated, resulting in its phosphorylation and the phosphorylation of insulin receptor substrate (IRS) proteins. The growth factor binding protein 2 (Grb2)-son of sevenless complex (SOS) can then be recruited directly along with Shc or via insulin receptor substrate 1 or 2 (2). Usually, this leads to activation of Ras, Raf, and the MEK1.

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The abbreviations used are: MEK, mitogen-activated protein kinase; Pip92, a proline-rich cytoplasmic protein that shares little homology to any known proteins but is another regulated immediate-early gene. Its mRNA is induced by serum in fibroblasts and pheochromocytoma cells (29, 30) and is increased in activated T-cells, and differentiated HL-60 cells (31, 32). We have previously shown that insulin regulates Pip92 mRNA abundance in rat hepatoma cells (33). Studies examining the mechanism of regulation of the Pip92 and c-Fos genes found that their induction in response to stimuli is dependent upon a serum-responsive element, in addition to other elements, within each gene (34, 35).

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FIG. 1. MEK1-dependent induction of ERK1/2 phosphorylation and kinase activity by insulin and p38 inhibitors. H4IIE cells were serum-deprived and treated for the indicated times, and protein was prepared and separated as described under "Experimental Procedures." A,
Little is known about interactions between the p38 and ERK1/2 MAPK signaling pathways. A few recent reports have suggested that some cross-talk may occur but with variable results. The data published to date indicate that any interplay between these two MAPK pathways may be specific to the cell type and the particular stimulus. Even less is known about the effects of the p38 pathway on insulin activation of ERK1/2. The present work describes cross-talk between the insulin-activated MEK/ERK pathway and the p38 pathway and the effects of the cross-talk on expression of two insulin-regulated immediate-early genes, c-Fos and Pip92. We identify the MEK/ERK pathway as a necessary component of insulin induction of these genes and demonstrate the ability of p38 inhibitors to modulate MEK/ERK signaling and insulin- and MEK/ERK-dependent gene expression.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Rat H4IIE hepatoma cells were obtained from ATCC (Manassas, VA) and maintained at 37 °C and 5% CO₂ in a humidified incubator. Serum deprivation (Sigma-Aldrich) supplemented with 2% fetal bovine serum, 3% calf serum, 5% horse serum (Invitrogen), 100 units/ml penicillin, and 100 µg/ml streptomycin (Mediatech, Herndon, VA). Serum was withdrawn from subconfluent cultures 24–48 h prior to experimental treatments. All of the insulin treatments (Sigma) were 10 nM. SB202190, SB202474, and SB203580 (Calbiochem, San Diego, CA) were used at 2 and 10 µM (50 µM) was obtained from Cell Signaling Technology (Beverly, MA), as were phosphorylated (active) ERK1/2, total ERK, and secondary rabbit horseradish peroxidase-conjugated antisera.

**Gene Expression**—The transcription rates were assayed by the nuclear run-on method (36). Briefly, transcriptionally active nuclei were labeled with [³²P]UTP (ICN, Costa Mesa, CA), RNA-isolated, and hybridized with cDNA spotted on nitrocellulose, and autoradiographed. Densitometric data from autoradiographs were analyzed using ZeroD Scan from Scancoffas. The rat Pip92 cDNA was cloned from a library of insulin-induced genes as previously described (33), and the complete murine c-Fos cDNA in pBR322 was obtained from the ATCC (pc-fos).

**Total and Active ERK1/2 Measurements**—Serum-deprived cultures were washed with 37 °C phosphate-buffered saline. Whole cell (SDS) lysates were prepared as previously reported (37) and were assayed for protein content using the Bio-Rad DC assay. The lysates (20 µg) were resolved by electrophoresis with NuPAGE gels and transferred to nitrocellulose membranes using the Mini Transblot apparatus (39, 40). Total proteins or phosphoproteins were visualized by ECL Plus (Amersham Biosciences) reagents and autodigographed. Densitometric data from three to six experiments were averaged.

**In Vitro Kinase Assays**—Nonradioactive kinase assay kits were obtained from Cell Signaling Technology and used according to the supplied protocols. Briefly, p38 and ERK kinases were purified by immuno- precipitation with specific immobilized antisera from 0.5–1.0 mg of soluble cell lysate by standard methods. The immune complex was washed twice in lysis buffer (1% Triton X-100, 10% glycerol, 20 mM Tris, pH 7.5, 150 mM NaCl, 7.5 µM aprotinin, 5 mM benzamidine, 5 mM phenylmethylsulfonyl fluoride, 50 mM NaF, and 0.125 mM NaVO₃) and then twice in kinase buffer (25 mM Tris, pH 7.5, 10 mM MgCl₂, 5 mM β-glycerophosphate, 0.1 mM NaVO₃, and 2 mM dithiothreitol). The immune complexes were then incubated at 30 °C for 30 min with 200 µM ATP and 2 µg of the purified recombinant substrate, GST-ATF-2 or GST-Elk, respectively (47–49). The kinase reaction was terminated by adding 0.1% SDS loading buffer and the phosphorylated substrate was detected by Western blotting with antisera specific for the phosphorylated form of ATF-2 or Elk1.

**Statistical Analysis**—Analysis of variance and Student’s t tests were performed using the Instat program (Graphpad Software Inc., San Diego, CA). The values from the experimental treatments were expressed as fold change compared with the vehicle-treated controls within each experiment. The relative strength of correlations was calculated using the Spearman rank correlation coefficient (rₛ).

**RESULTS**

**Insulin, MEK/ERK, and p38 Signaling Cross-talk**—To directly characterize the intracellular signaling pathways activated by insulin in rat hepatoma cells, Western blots were performed using antisera to total and phosphorylated ERK1/2 (P-ERK1/2). As expected, treatment of H4IIE cells with insulin caused a large and rapid increase in phosphorylation of both ERK1 and ERK2 (Fig. 1A, upper panel). Pretreatment of cells with the MEK 1 inhibitor (PD98059 (PD)) abolished the insulin effect on ERK1/2 phosphorylation at all time points tested (Fig. 1A).

Studies were performed to investigate the cross-talk between the MEK/ERK and p38 signaling pathways. To determine whether regulation of p38 was involved in acute insulin activation of ERK1/2, serum deprived cells were pretreated with the p38 inhibitor SB202190 (SB) at concentrations of 2 or 10 µM over a period of 5–270 min and compared with control (vehicle-treated) cells. Western blots revealed that, by itself, 2 µM SB, and to a greater extent 10 µM SB, induced ERK1/2 activation in a time-dependent manner (P-ERK1/2; Fig. 1, B and C). The levels of total ERK1/2 immunoreactive protein were not significantly or consistently altered by any of the treatments (Fig. 1, A–C, lower panels), indicating that changes in P-ERK1/2 bands reflected changes in the fraction of activated kinase. The return to baseline was more rapid with SB, with no apparent rise in bands at each time of SB treatment and expressed as the average fold increases of band intensity compared with untreated control cell lysate within each experiment (Fig. 1D). Treatment with 2 µM SB resulted in a maximum of 15.6-fold induction of P-ERK1/2 over control by 30 min compared with 33-fold above control by 60 min in cells treated with 10 µM SB. For both concentrations of SB, P-ERK1/2 remained elevated above control levels even at 150 and 270 min, the longest time points tested.

Studies were performed to ensure that our measurements of P-ERK1/2 by Western blot were linear within the range of proteins used and the amount of P-ERK1/2 generated. Using a highly sensitive digital video camera, chemiluminescent Westerns were imaged directly. Over a 1–30-fold range, P-ERK1/2 band intensity in Western blots correlated with whole cell lysate concentration and, thus, P-ERK1/2 concentration in a linear fashion (Fig. 1E).
In addition, to ensure that insulin-induced phosphorylation of ERK1/2 correlated well with ERK activity, we performed ERK1/2 kinase assays (Fig. 1F). We found an identical pattern of ERK1/2 activity compared with ERK1/2 dual phosphorylation (P-ERK1/2) as described previously (50, 51). Because there was such consistency between P-ERK1/2 and ERK1/2 activity, the simpler (P-ERK1/2) assay was used throughout the remainder of the studies.

To examine whether the p38 inhibitor alters the ability of insulin to activate ERK1/2, H4IIE cells were pretreated with either 2 or 10 μM SB for 30 min. The cells were then either harvested immediately or treated with insulin for 5–240 min.
Pretreatment with SB alone caused the expected, concentration-dependent large increase in P-ERK1/2. In addition, the induction of P-ERK1/2 was greatly augmented by insulin treatment (Fig. 2A and B). Insulin treatment alone caused a maximum increase in ERK1/2 phosphorylation of ~28-fold by 5 min followed by a decrease to a plateau of around 2-3-fold above control levels between 120 and 240 min (Fig. 2C, left panel).

Pretreatment with the lower concentration of SB (2 µM) caused an approximate doubling of the maximum level of insulin-induced P-ERK1/2 (28 versus 59-fold), and the higher concentration of SB (10 µM) caused a ~4-fold increase in maximum ERK1/2 phosphorylation compared with insulin alone (110-fold above serum free control). These increases in response to insulin are in addition to the elevated P-ERK1/2 at the beginning of insulin treatment that results from the 30-min incubation with SB. For comparison, in the right panel of Fig. 2C is the effect of SB alone (replicated from Fig. 1C, but on the expanded scale necessary for these experiments) or the combination of SB and insulin as presented in the left panel. Regulation of ERK activity in vitro kinase assays parallels the induction of P-ERK1/2 in H4IIE cells treated with SB alone or prior to insulin addition (Fig. 2D).

This indicates a greater than additive effect of 2 and 10 µM SB in combination with insulin to induce P-ERK1/2 as compared with the effects of the agents added separately (Fig. 2C). For example, if the effects are compared (treatment with SB 2 µM plus insulin versus the addition of the effects of SB 2 µM alone plus the effects of insulin alone), at 5, 15, and 120 min, there were 42, 122, and 266% increases above additivity, respectively, for induction of P-ERK1/2, suggestive of a synergistic effect of the combination of low dose SB and insulin. The effect of pretreatment with the higher dose of SB (10 µM), followed by insulin addition resulted in 81, 161, and 266% increases above additivity for induction of P-ERK1/2 at 5, 15, and 120 min, respectively, above the simple addition of the effects of 10 µM SB alone and insulin alone. Throughout all of these experiments, there were no significant changes in the amount of total ERK1/2 protein, indicating that changes in the phosphorylation of ERK1/2 reflected changes in the fraction of activated kinase (Fig. 2A and B, lower panels).

To ensure that the effects of p38 inhibition by SB are not a unique feature of this compound, a separate but chemically similar inhibitor was also tested. The distinct inhibitor, SB203580, was also found to induce ERK1/2 phosphorylation when added alone (Fig. 3A, lane 1). SB203580 also acted synergistically with insulin to increase ERK1/2 activation (Fig. 3A, lanes 2-4). As a further control, a structurally related but inactive compound, SB202474, which does not inhibit p38 kinase activity, was also tested on H4IIE cells and was unable to

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**Fig. 3.** Effect of different SB compounds on ERK1/2 phosphorylation alone and in combination with insulin. H4IIE cells were serum-deprived and then left untreated or pretreated for 30 min prior to treatment for the indicated times with insulin (Ins). Shown are representative autoradiographs of Western blots of whole cell lysates probed with anti-P-ERK1/2 as described under “Experimental Procedures.” A, cells were pretreated with 2 µM SB203580 prior to treatment with 10 nM insulin for the indicated times. B, cells were pretreated with SB202474 or SB202190 at the indicated concentrations alone (−) or prior to treatment (+) with 10 nM insulin for 5 min.

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**Fig. 4.** Effect of MEK1 inhibition on induction of c-Fos and Pip92 transcription by insulin. H4IIE cells were serum deprived and then treated for 30 min with 10 nM insulin (Ins 30) alone or after pretreatment with 50 µM PD98059. Transcriptionally active nuclei were then isolated and used in run-on assays. A, representative autoradiograph of a run-on experiment as described under “Experimental Procedures,” with cells treated as indicated. The results graphed in B are the averages of four to eight separate experiments with error bars (S.E.). The dot densitometric intensity for the control was arbitrarily set to 1 in each experiment, and other treatment values were expressed as fold increase versus control. #, greater than control (p < 0.001); §, decreased from insulin alone (p < 0.001).
increase ERK1/2 phosphorylation on its own or in combination with insulin treatment (Fig. 3B).

Insulin, MEK/ERK, and p38 Regulation of Gene Expression—To examine the effects of activation of the ERK1/2 pathway and the possible cross-talk of the ERK and p38 pathways in insulin regulation of gene expression, nuclear run-on assays were performed. H4IIE cells were either not treated or treated with 10 nM insulin, and the transcription rate of c-Fos and Pip92 was measured. In addition, other cells were pretreated either with vehicle (control) or with the MEK1 inhibitor (PD) to inhibit signaling through the MEK1/ERK1/2 pathway. As we have previously reported (27, 33), following 30 min of insulin treatment, there were significant increases in transcription of both genes (Fig. 4). There was no significant effect on the transcription of either gene when cells were treated with PD alone (50 μM). In cells pretreated with PD, the effects of insulin on c-Fos and Pip92 were reduced –70–80%, to levels not significantly different from basal (control) levels of transcription. These results indicate that MEK1, and most likely ERK1/2 activation, is necessary for the full effect of insulin-mediated induction of both c-Fos and Pip92 genes. A control gene, β-tubulin, was not significantly altered by insulin or PD alone or by the combination of the two.

We then determined whether regulation of p38 could effect insulin-mediated regulation of c-Fos and Pip92 transcription. Serum-deprived cells were pretreated with the p38 inhibitor (SB) at concentrations of 2 or 10 μM and compared with vehicle control. Following a pretreatment period, the cells were stimulated acutely with 10 nM insulin for 30 min or left untreated. Low dose (2 μM) SB alone increased c-Fos transcription 5.5-fold while having a slight but nonsignificant effect on Pip92 (Fig. 5A; insulin data from Fig. 4B added to this graph for comparison). Pretreatment with 2 μM SB followed by stimulation with insulin for 30 min resulted in a 26.5-fold increase in c-Fos and a 12.2-fold induction of Pip92 transcription (Fig. 5A). For the two induced genes, the effect of 2 μM SB alone was less than that of insulin (48% for c-Fos and 21% for Pip92), whereas the combination of insulin and SB on transcription is greater than additive. If the effects are compared (treatment with SB 2 μM plus insulin versus the addition of the effects of SB 2 μM alone plus the effects of insulin alone), there was a 55% increase above additivity for c-Fos and a 61% increase above additivity for Pip92, suggestive of a synergistic effect (Fig. 5A), similar to that found in P-ERK1/2 induction. There was no significant effect of any of these treatments on transcription of the control gene, β-tubulin.

The higher dose of SB (10 μM) by itself caused a dramatic 36-fold increase in c-Fos transcription, much larger than the 11.5-fold effect of insulin alone (the insulin data is again shown in Fig. 5B for comparison). Unlike the lower dose of SB, which was ineffective, the 10 μM concentration of SB induced Pip92 transcription 5.5-fold, similar to the 6.3-fold effect of insulin alone. When H4IIE cells were pretreated with the higher dose of SB and were then exposed to insulin for 30 min, there was no further increase in c-Fos transcription compared with 10 μM SB alone. However, this regimen resulted in an induction of Pip92 transcription that is 83% larger (21.5-fold) than the sum of the effects of 10 μM SB alone (5.5-fold) and insulin alone (6.3-fold), again suggesting a synergistic interaction of insulin and SB. The synergistic effects of SB and insulin on gene transcription coincided well with the synergistic effects of the p38 inhibitor and insulin on the induction of P-ERK1/2.

Previous studies from our laboratory (27) have shown that other potent inducers or combinations of inducers of immediate-early gene expression cause a maximum induction of 35–45-fold in c-Fos transcription in H4IIE cells and could never

![Fig. 5. MEK1-dependent effect of p38 inhibition on induction of c-Fos and Pip92 transcription and ERK1/2 phosphorylation.](image)
induce transcription beyond the 35–45-fold that is also observed in the present experiments. This suggests that the increase in transcription induced by 10 μM SB (alone) may achieve the maximum possible induction of c-Fos transcription in H4IIE cells. Thus, the combination of SB and insulin could not cause a further increase.

It is clear that the addition of SB can increase phosphorylation of ERK1/2 and result in the increased transcription of insulin-regulated, ERK-dependent genes. Additionally, there is a synergistic induction of P-ERK1/2 and the ERK-dependent genes, c-Fos and Pip92, when SB and insulin are combined. However, whether a common pathway was used by both insulin and p38 inhibitors to activate ERK1/2 and subsequently induce c-Fos and Pip92 expression was tested by again using the MEK1 inhibitor, PD98059. After 30 min of pretreatment with PD, the cells were treated or not for a further 30 min with SB. Greatly overexposed Western blots (Fig. 5C) indicated that the MEK1 inhibitor completely blocked SB-induced increases in ERK1/2 phosphorylation, as well as slightly reducing basal phosphorylation, suggesting that MEK1 activity is required for the activation of ERK1/2 by the p38 inhibitor.

When the effects of MEK1 inhibition were studied on the stimulation of c-Fos and Pip92 expression, there was an inhibition of the SB (10 μM) induction of both genes by PD pretreatment (Fig. 5B). In addition, when cells were pretreated with PD prior to the combination of SB and insulin, the large induction of both c-Fos and Pip92 transcription were completely inhibited (Fig. 5B). This suggests that SB induces transcription of these ERK1/2-dependent genes, acting at the level of MEK1 or at a point in the insulin signaling pathway upstream of MEK1.

The similarities in the induction of ERK1/2 activation/phosphorylation and transcriptional regulation of both c-Fos and Pip92 was striking. To further examine the relationship between these two variables, the correlation was quantitated (52). Linear regression lines were obtained for induction of each gene (represented as the y variable) and the corresponding level of P-ERK induction (represented as the x variable) for each treatment described. The slopes of the lines obtained for c-Fos and Pip92 were 0.32 and 0.21, respectively (Fig. 6). The strengths of the correlation described by these regression lines ($r_s = 0.9084$ and 0.8772, respectively) and their significance ($p < 0.0001$) (53) further support the idea that insulin alone, p38 inhibitor alone, or the combination of treatments all regulate c-Fos and Pip92 via ERK1/2.

We then measured whether insulin had a direct effect on p38 activity in H4IIE cells. Consistent with our cross-talk hypothesis, basal activity of p38 was observed in quiescent cells. A modest, but not statistically significant increase in p38 activity was sustained over a time course of insulin treatment at all times greater than 5 min (Fig. 7). Small changes in p38 activity in response to insulin are consistent with recent reports of studies in a cultured skeletal muscle cell line (10).

**DISCUSSION**

To study the regulation of insulin signaling, the activation (phosphorylation) state of ERK1/2 was examined in rat H4IIE hepatoma cells. Insulin treatment alone resulted in a rapid increase in ERK1/2 phosphorylation and activity, whereas pretreatment with the MEK1 inhibitor, PD98059, abolished the effect of insulin on P-ERK1/2 as well as insulin induction of the c-Fos and Pip92 genes, implying that insulin control of these genes proceeded primarily through the MEK1-ERK1/2 pathway.

Our findings agree with work in rat hippocampal neurons...
indicating that Raf- or fibroblast growth factor-mediated activation of ERK1/2 via MEK1 is required for the rapid activation of Pip92 transcription (34). In 3T3-L1 adipocytes, insulin induction of a c-Fos reporter construct was prevented by MEK1 inhibition (54). Thus, MEK/ERK activation is important for the activation of these genes and, as presented here, is of specific importance for insulin induction of c-Fos and Pip92 in rat H4IE hepatoma cells.

When H4IE cells were treated with SB alone, there was a dose-dependent induction of P-ERK1/2 and of c-Fos and Pip92 transcription. Further, when cells pretreated with SB were given a secondary insulin stimulus, there was a greater-than-additive activation of ERK1/2 and of c-Fos and Pip92 transcription. It is not entirely clear why the lower concentration of SB alone is more potent with respect to transcription of c-Fos than Pip92, but presumably there are other factors involved in c-Fos and Pip92 transcription because they are not uniformly induced in these studies. One possibility is that Pip92 has a higher “threshold” of ERK1/2 activity necessary for induction than does c-Fos.

The activation of ERK1/2 observed by treatment of cells with the p38 inhibitor SB202190 was also observed when cells were treated with a different p38 inhibitor, SB203580. The chemically related compound SB202474, which does not inhibit p38, has no effect on ERK1/2 phosphorylation either alone or in combination with insulin. This implies that it is the inhibition of p38 itself and not a nonspecific action of one of the p38 inhibitors that results in induction of P-ERK1/2.

One could hypothesize that p38 activity inhibits transcription through a mechanism separate from the ERK1/2 pathway used by insulin in the regulation of these two genes and that the synergistic actions of the p38 inhibitors and insulin were due to SB having effects downstream of ERK1/2 activity. Thus, when p38 was inhibited by SB, this separate inhibitory pathway would have been neutralized, and the c-Fos and Pip92 genes would be induced to a greater degree. Clearly, this hypothesis was incorrect because the p38 inhibitors resulted in activation of ERK1/2 and acted to synergistically induce P-ERK1/2 in combination with insulin.

Another hypothesis initially considered was that basal p38 activity was responsible for a separate and distinct “off” signal for transcription. In this model, transcription would be turned on by insulin, but the off signal would be deficient in the presence of p38 inhibition, and transcription would continue unchecked. This also was not supported by the evidence, because the p38 inhibitors alone could induce P-ERK1/2 and transcription of the c-Fos and Pip92 genes, even in the absence of insulin.

Alternatively, the p38 pathway may have a tonic, inhibitory effect on MEK/ERK signaling, and inhibition of p38 by SB increased ERK1/2 pathway activity resulting in induction of ERK1/2-dependent genes. When a stimulator of the MEK/ERK pathway was added in combination with the p38 inhibitors, a greater fold induction of P-ERK1/2 and the c-Fos and Pip92 genes was obtained. This is a potential explanation of the data obtained in the present work, because the addition of an active, but not the inactive p38 inhibitor by itself resulted in activation of ERK1/2 signaling and subsequent transcription of c-Fos and Pip92. Our data also suggested that the activation occurs at or above the level of MEK1, because inhibition of MEK1 activity blocked the induction of transcription and P-ERK1/2 by insulin alone, SB alone, and the combination of SB and insulin. Conversely we found the basal level of p38 activity is modestly induced by insulin. The fact that this occurs with a slightly delayed time course, corresponding to the downward slope of the peak of insulin-induced ERK1/2 activity, fits well with the idea that p38 may play a negative regulatory role.

Data regarding the activation of other, parallel, MAPK pathways by insulin has been somewhat contradictory. However, recent studies have indicated a major role of the p38 signaling pathway in insulin-inducible glucose transport. In these studies, insulin induced p38 activity in L6 myotubes. The p38 inhibitors, SB202190 and SB203580, reduced insulin-stimulated glucose transport by 40–60% without altering insulin-induced Akt phosphorylation (8–11). In the present studies, the same inhibitors activated ERK and worked synergistically with insulin to stimulate ERK-dependent genes. This suggests a potential complex role of the p38 signaling pathway in the multiple actions of insulin. This may also imply a role for p38 that varies in the different insulin target tissues.

Several recent reports suggest possible cross-talk between the MEK/ERK and p38 pathways, in ways supportive of our findings. For instance, in PANCl-1, HL-60, L1210, and ECV304 cells, ERK1/2 activity or phosphorylation is increased by SB203580 or SB202190 (55–58). In human skin fibroblasts, phorbol ester- and Raf-mediated induction of MEK1 and ERK1/2 is inhibited when p38 is activated by constitutively active MAPK kinase 3b, the kinase upstream of p38 (59), and in the human hepatoma cell line, HepG2, low density lipoprotein receptor mRNA and P-ERK1/2 are increased by SB treatment and reduced by constitutively active MAPK kinase 6b, another kinase upstream of p38 (60). These observations suggest a role for the p38 pathway in MEK-ERK-dependent processes.

However, several other reports obtained results different from those presented here; in baboon smooth muscle cells and Swiss 3T3 fibroblasts use of the p38 inhibitor activates Raf, the upstream kinase for MEK1, but somehow fails to activate MEK1 and ERK1/2 (61, 62). Treatment with SB203580 did not alter epidermal growth factor-induced ERK activation in Swiss 3T3 cells or IGF-1-induced ERK activation in L6 myotubes (62), and in mIMCD3 cells, inhibition of p38 blocks activation of P-ERK1/2 by hypertonic NaCl treatment (63). Thus, there is extensive heterogeneity in the nature of the interactions between the MEK-ERK and p38 pathways. There appear to be cell type differences, differences in interactions with various stimulators of intracellular signaling, and differences in the effects of p38 activation or inhibition on the downstream effects being measured following activation of the MEK-ERK pathway.

The present studies have identified potential cross-talk between the MEK-ERK and p38 pathways and a role of this interaction in insulin signaling and insulin action. The modest and slightly delayed effect of insulin on p38 activity may indicate that it functions to limit ERK1/2 activation and subsequent gene transcription rather than actively decreasing ERK1/2 activity and transcription. Future studies are required to identify the target at or upstream of MEK1 in the MEK-ERK signaling pathway affected by addition of p38 inhibitors, and why these effects may be tissue-specific. Future experiments will also be needed to determine whether insulin regulation of other insulin-sensitive genes is modulated by changes in p38 activity and subsequent changes in MEK1/ERK signaling.

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