Regulation of Mitogen-activated Protein Kinase Phosphatase-1 Induction by Insulin in Vascular Smooth Muscle Cells

EVALUATION OF THE ROLE OF THE NITRIC OXIDE SIGNALING PATHWAY AND POTENTIAL DEFECTS IN HYPERTENSION

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In this study, we examined the regulation of mitogen-activated protein kinase phosphatase (MKP-1) expression by insulin in primary vascular smooth muscle cell cultures. Insulin caused a rapid time- and dose-dependent induction of MKP-1 mRNA and protein expression. Blockade of nitric-oxide synthase (NOS) with L-monomethyl-L-arginine acetate, and cGMP with RpeGMP, completely inhibited MKP-1 expression. Insulin-mediated MKP-1 expression was preceded by inducible NOS (iNOS) induction and cGMP production. Blockade of phosphatidylinositol (PI3-kinase) signaling with wortmannin inhibited insulin-mediated iNOS protein induction, cGMP production, and MKP-1 expression. To evaluate potential interactions between NOS and the mitogen-activated protein kinase (MAPK) signaling pathways, we employed PD98059 and SB203580, two specific inhibitors of ERKs and p38 MAPK. These inhibitors abolished the effect of insulin on MKP-1 expression. Only PD98059 inhibited insulin-mediated iNOS protein induction. Vascular smooth muscle cells from spontaneous hypertensive rats exhibited a marked decrease in MKP-1 induction due to defects in insulin-induced iNOS expression because of reductions in PI3-kinase activity. Treatment with sodium nitroprusside and 8-bromo-cGMP restored MKP-1 mRNA expression to levels comparable with controls. We conclude that insulin-induced MKP-1 expression is mediated by PI3-kinase-initiated signals, leading to the induction of iNOS and elevated cGMP levels that stimulate MKP-1 expression.

Intracellular signaling following stimulation by insulin, other growth factors, tumor promoters, cytokines, osmotic shock, and stress involves the initiation of one or more phosphorylation cascades leading to the rapid and reversible activation of a family of ubiquitous, well characterized serine/threonine kinases known collectively as mitogen-activated protein kinases (MAPKs, 1–4). MAPKs play a major role in regulating cellular events required for cell growth, differentiation, and cell homeostasis (1–4). Three major subclasses of MAPKs have been identified recently and comprise the ERK, SAPK/JNK, and p38HOG families (1–4, 5). Full activation of MAPKs requires phosphorylation on critical tyrosine and threonine residues. Several upstream dual-specificity kinases catalyzing this modification have been identified (6). Once activated, these kinases are responsible for the activation and phosphorylation of additional kinases as well as a battery of regulatory proteins, including transcription factors required for the expression of genes involved in cell growth and/or differentiation (6).

The activities of all the three members of the MAPK family are regulated by reversible phosphorylation of tyrosine and threonine residues, indicating that protein kinases play a critical role in controlling enzyme activity. Recent studies indicate that inactivation or attenuation of MAPK signaling is mediated by a class of dual specificity protein kinases (7–8). These include MKP-1 (also known as CL100, Erp, and hVH-1, which is encoded by murine gene, 3ch134 (9), MKP-2, MKP-3, PAC-1, and B23 (10). MKP-1, the most ubiquitously expressed and best studied of these phosphatases, has dual catalytic activity toward phosphotyrosine- and phosphothreonine-containing proteins and is known to inactivate ERKs, JNK, and p38HOG in vivo as well as in vitro (7, 11). MKP-1 and the other family members are principally regulated at the transcriptional level as evidenced by very low to undetectable mRNA expression in quiescent cells and a rapid induction upon treatment of cells with growth factors, as well as agents that cause oxidative stress and heat shock (7, 11). MKP-1 has been implicated in a feedback loop serving to inactivate MAPK after stimulation by mitogens as well as during the cellular response to stress (7, 11–12). Despite its high level of induction following treatment with FBS and angiotensin II (13), a function for this phosphatase in response to insulin or IGF-1 has not been established in vascular smooth muscle cells (VSMCs).

Hypertension is frequently associated with insulin-resistant states such as diabetes and obesity (14–15). However, mechanisms linking hypertension with insulin resistance are not clear. VSMCs are a major constituent of blood vessel walls responsible for the maintenance of vascular tone (16). Accelerated VSMC growth, hypertrophy, and abnormal vascular tone play a central role in the development of hypertension (17–18). Although alterations in insulin action of the vasculature have been proposed to contribute to atherosclerosis and the regulation of vascular tone, little is known about the pathways of insulin signaling that control vascular tone and cell growth or

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1 The abbreviations used are: MAPK, mitogen-activated protein kinase; MKP-1, mitogen-activated protein kinase phosphatase; VSMC, vascular smooth muscle cells; NOS, nitric-oxide synthase; iNOS, inducible NOS; PI3-kinase, phosphatidylinositol 3-kinase; L-NMMA, Nω-monomethyl-arginine acetate; RpeGMP, Rp-8 CPT-cyclic guanosine monophosphate; IGF-1, insulin growth factor-1; FBS, fetal bovine serum; SHR, spontaneous hypertensive rats; SNP, sodium nitroprusside; WKY, Wistar Kyoto rats; ERK, extracellular regulated kinase; JNK, c-jun amino-terminal kinase; SAPK, stress-activated protein kinase.
the mechanism of their regulation in VSMCs.

Elegant studies by Baron and co-workers (19) have shown that insulin is a potent vasodilator, and this effect of insulin is mediated by nitric oxide (NO, see Ref. 19). NO is also known to influence the growth of VSMCs. However, the mechanism of NO action remains unclear.

We have recently shown that confluent primary cultures of VSMCs isolated from spontaneous hypertensive rats (SHR) exhibit increased responsiveness to insulin in terms of MAPK activation and DNA synthesis (20). It is not known whether the observed increase in MAPK activation in SHR is due to defective regulation of MKP-1 mRNA induction resulting from alterations in intracellular insulin signaling pathways that mediate MKP-1 expression or unstable MKP-1 mRNA.

In this study, in order to gain insight into potential mechanisms linking insulin signal transduction with hypertension, we examined the kinetics of MKP-1 induction by insulin and evaluated the contribution of PI3-kinase, nitric oxide, and cGMP signaling pathway(s) in insulin regulation of MKP-1 expression using primary cultures of VSMCs isolated from SHR and normotensive Wistar Kyoto rats (WKY). The results of this study indicate that insulin increases MKP-1 mRNA expression in VSMCs mainly via the PI3-kinase/NO-cGMP signaling pathway, and the observed defects in MKP-1 expression in SHR are due to defective PI3-kinase/NOS signaling leading to reductions in cGMP which may mediate MKP-1 gene expression.

**EXPERIMENTAL PROCEDURES**

**Materials**—Minimal essential medium-α, fetal bovine serum (FBS), antibiotics, trypsin, t-glutamine, and freezing medium were obtained from Life Technologies, Inc., α-[32P]ATP (specific activity, 3000 Ci/m mole), and [33P]ATP (specific activity, 2000 Ci/m mole), and 125I-protein A were purchased from NEN Life Science Products. Type 1 collagenase was from Worthington. Antibodies against MKP-1 and iNOS were purchased from NEN Life Science Products. Type 1 collagenase. The MKP-1 mRNA and SERCA mRNA induction by insulin is comparable with the previously reported kinetics with 10% FBS and angiotensin II (13). Alkaline phosphatase activities were quantitated by densitometric analyses. We have recently shown that confluent primary cultures of VSMCs isolated from spontaneous hypertensive rats (SHR) exhibited an approximate 2-fold increase in MAPK activation in SHR is due to defective regulation of MKP-1 mRNA expression in VSMCs mainly via the PI3-kinase/NO-cGMP signaling pathway, and the observed defects in MKP-1 expression in SHR are due to defective PI3-kinase/NOS signaling leading to reductions in cGMP which may mediate MKP-1 gene expression.

**RESULTS**

**Differential Effect of Insulin on MKP-1 mRNA Induction in VSMCs Isolated from WKY and SHR**—To understand the mechanism of sustained MAPK activation in SHR, we examined the effect of insulin on MKP-1 induction. As seen in Fig. 1A, low levels of MKP-1 mRNA are expressed in VSMCs in the basal state in both WKY and SHR. Insulin as well as IGF-1 treatment for 30 min caused a 100% increase over basal levels in MKP-1 mRNA expression in WKY. In contrast, VSMCs from SHR exhibited a lower basal as well as a significant reduction in insulin/IGF-1-mediated MKP-1 mRNA expression (Fig. 1). Western blots revealed a 1.5-fold increase in MKP-1 mRNA induction in VSMCs treated with insulin revealed a 2.5-fold increase after 10 min in WKY (Fig. 2A). The stimulation was sustained for 30 min with a return toward basal levels in 60 min. The time course of MKP-1 mRNA induction by insulin is comparable with the previously reported kinetics with 10% FBS and angiotensin II (13). Although VSMCs from SHR exhibited an approximate 2-fold increase in MKP-1 mRNA induction over the basal levels after 10 min of insulin treatment, the stimulation was not sustained for longer periods. This may be due to instability of the mes-
MKP-1 expression. The signaling through these pathways was blocked by pretreatment of VSMCs with selective inhibitors of PI3-kinase, NO, and cGMP, the downstream effector of NO.

Fig. 1. Differential effect of insulin and IGF-1 on MKP-1 mRNA expression in VSMCs isolated from WKY and SHR. Serum-starved VSMCs from WKY and SHR were treated with insulin (100 nM) or IGF-1 (10 ng/mL) for 30 min followed by extraction of RNA. Equal amount of RNA (10 µg/well) was separated on agarose-formaldehyde gel followed by overnight transfer to nitrocellulose membrane. The membranes were hybridized with 32P-labeled MKP-1 cDNA probe, stripped, and reprobed with a β-actin probe. A representative autoradiogram is shown. Bottom panel, quantitation of MKP-1 mRNA levels from multiple experiments by densitometric analyses. The relative mRNA levels were determined by laser densitometric scanning of the autoradiograms. To correct for variations in RNA loading, the intensity of MKP-1 signal was divided by the intensity of β-actin signal. The results are expressed as arbitrary densitometric units. To compare results from different experiments, mRNA from WKY control was assigned a value of 1, and the rest of the data was calculated relative to the WKY control. Results are the mean ± S.E. of four separate RNA blots from different experiments. * and ** denote p < 0.05 versus WKY control; *** versus insulin/IGF-1-treated WKY.

As expected, inhibition of insulin receptor tyrosine kinase activation by pretreatment with erbstatin A or herbimycin A completely blocked the subsequent effect of insulin on MKP-1 mRNA expression (data not shown). NO donor and cGMP antagonist did not block FBS-induced MKP-1 expression, suggesting that multiple signaling pathways may be involved in MKP-1 expression. Both the inhibitors also caused a modest decrease in basal expression of MKP-1 mRNA (data not shown).

VSMCs express inducible form of NO (iNOS, see Ref. 33). To evaluate the role of the NO signaling pathway in insulin-mediated MKP-1 induction and to identify potential defects in this pathway in SHR further, we measured the kinetics of iNOS protein induction and cGMP generation in insulin-stimulated VSMCs. As seen in Fig. 4A, insulin caused a rapid time-dependent increase in iNOS protein expression in WKY (2–3-fold increase over basal levels). Maximal increase in iNOS protein expression was observed after 5 min, and the effect was sustained for 30 min and returned to basal levels after 1 h. In contrast, VSMCs from SHR were resistant to insulin as evidenced by a lack of insulin-induced iNOS protein expression (Fig. 4A). Inhibition of PI3-kinase with wortmannin blocked the stimulatory effect of insulin on iNOS protein expression in WKY (Fig. 4B).

Insulin-mediated iNOS protein induction was accompanied by a time-dependent increase in cGMP levels. A >3-fold increase in cellular cGMP levels was observed after 10 min of exposure to insulin with a return to basal levels in 60 min (Fig. 5A). VSMCs from SHR exhibited a 40% reduction in basal cGMP levels and a marked impairment (>80%) in cGMP production in response to insulin when compared with WKY (Fig. 5, A and B). However, SNP-induced cGMP production was comparable between WKY and SHR (Fig. 5B). This confirms that the defect in SHR is at the level of NO production and not due to defective guanylyl cyclase activity. Inhibition of PI3-kinase with wortmannin and iNOS with l-NMMA completely prevented insulin-mediated cGMP production (Fig. 5B). Wortmannin and LNMMA did not block SNP-mediated cGMP production (data not shown).

Effect of Insulin on PI3-Kinase Activation in SHR and WKY—Our results on the inhibitory effects of wortmannin on insulin-mediated MKP-1 and iNOS induction suggested that PI3-kinase signaling may mediate the effects of insulin on iNOS induction, cGMP production, and MKP-1 expression. To evaluate further whether insulin differentially activates PI3-kinase in WKY and SHR, we measured PI3-kinase activity in
IRS-1 immunoprecipitates. As seen in Fig. 6, A and B, insulin rapidly stimulates IRS-1-associated PI3-kinase activity in a dose-dependent manner in both cell types. In WKY, 10–100 nM insulin caused a 2–3-fold increase in IRS-1 associated PI-3 kinase activity. A maximal stimulation (≈6 fold over basal) of IRS-1-associated PI3-kinase was observed with 1000 nM insulin. When compared with WKY, SHR exhibited 50–70% decrease in PI3-kinase activity at all insulin concentrations tested, although fold activation over basal value was comparable between the two cell types.

Analyses of Interaction between NOS and MAPK Signaling Pathways—A number of recent studies in different cell types suggest that MKP-1 induction is regulated by MAPK family members (7, 34–36). Therefore, to evaluate the potential contribution of MAPK signaling pathway in insulin-mediated iNOS and MKP-1 induction, cells were pretreated with PD98059 (a specific MEK inhibitor to inhibit ERKs, Ref. 20) and SB203580, a specific inhibitor of p38HOG MAPK, for 30 min followed by insulin exposure for 30 min. The isolated RNA was subjected to Northern blot analysis. A representative autoradiogram is shown. Similar results were obtained in three separate experiments. GADPH, glyceraldehyde-3-phosphate dehydrogenase.

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Insulin Induces MKP-1 via the Nitric Oxide Signaling Pathway—Insulin rapidly stimulates the induction of iNOS protein in WKY. Defective MKP-1 expression in SHR is accompanied by impaired iNOS protein induction. VSMCs were treated with 100 nM insulin for indicated times followed by extraction of protein in lysis buffer containing 1% Triton X-100. Equal amounts of proteins (50 μg) were separated on 7.5% denaturing gel followed by Western blot analyses with a polyclonal iNOS antibody. A representative autoradiogram is shown. Similar results were obtained in four separate experiments. B, wortmannin blocks insulin-induced expression of iNOS in WKY. VSMCs were treated with 100 nM wortmannin for 30 min followed by insulin treatment for 30 min. Cell lysates were examined for iNOS as detailed above. A representative autoradiogram is shown.
and B, PD98059 completely blocked insulin-mediated expression of iNOS and prevented the subsequent effect of insulin on MKP-1 expression. As reported in our recent publication (20), in addition to the observed inhibition of iNOS and MKP-1, PD98059 also blocked growth stimulatory effects of insulin in VSMCs by inhibiting insulin-mediated MAPK activation (20). In contrast, SB203580, a p38HOG kinase inhibitor, did not affect insulin-induced iNOS but inhibited MKP-1 expression to below basal levels (Fig. 7B). These observations together with the fact that insulin does not affect p38HOG MAPK activity suggest that ERKs may regulate NOS and MKP-1 induction, but the other MAPK family members may coordinate with ERKs in regulating MKP-1 using additional unidentified signaling pathways.

**DISCUSSION**

The results presented in this study clearly indicate that low concentrations of insulin rapidly induce MKP-1 mRNA and protein expression in VSMCs. The insulin-induced MKP-1 mRNA expression appears to be transient with a return toward basal levels within 60 min. The time course of MKP-1 induction by insulin parallels our recently reported kinetics of MAPK inactivation in VSMCs isolated from WKY (20). Thus, MKP-1 may act as an inhibitory feedback signal in attenuating MAPK signaling in VSMCs.

Several lines of evidence presented in this study suggest that MKP-1 induction by insulin in VSMCs is mediated via PI3-kinase-initiated NOS-cGMP signaling pathway. First, inhibition of PI3-kinase with wortmannin blocked insulin-mediated iNOS, cGMP, as well as MKP-1 mRNA induction. Second, blockade of NOS activity with L-NMMA inhibited the effect of insulin on MKP-1 mRNA induction. Third, treatment with RpcGMP, a cGMP antagonist, prevented insulin-induced MKP-1 mRNA expression. Finally, treatment with cGMP agonist, 8-bromo-cGMP, as well as SNP, a NO donor, mimicked the effect of insulin on MKP-1. Moreover, the effects of SNP, 8-bromo-cGMP, and insulin were not additive. More importantly, the NOS-cGMP signaling pathway appears to be dependent upon MAPK signaling as inhibition of ERKs completely blocks iNOS induction by insulin. These results suggest a complex cross-talk between ERKs and NOS-signaling pathway.
Insulin Induces MKP-1 via the Nitric Oxide Signaling Pathway

Given the knowledge that insulin acts as a potent vasodilator and increases the production of NO in endothelial cells (38), we examined the contribution of the NO signaling pathway in insulin-mediated iNOS induction. Insulin caused a rapid time- and dose-dependent increase in IRS-1-associated PI3-kinase activity. Treatment with wortmannin, a potent PI3-kinase inhibitor, completely blocked the stimulatory of insulin on MAPK activation and cell growth as wortmannin blocked the stimulatory of insulin on MAPK activation and mitogenesis (20). Thus, it appears from the current study as well as from our previous results that insulin-mediated PI3-kinase activation leads to activation of distinct signal transduction pathways that mediate MAPK activation and cell growth as well as MKP-1 induction to terminate MAPK signaling.

VSMCs from SHR exhibited marked reductions in PI3-kinase activity, iNOS induction, and cGMP production in response to insulin. However, the effects of cGMP agonist and SNP on MKP-1 induction were preserved in SHR. These observations suggest that the impaired induction of MKP-1 in SHR is due to defects upstream of NO, at the level of PI3-kinase/PI3-kinase activation. Insulin caused a rapid time- and dose-dependent increase in IRS-1-associated PI3-kinase activity. Treatment with wortmannin, a potent PI3-kinase inhibitor, completely blocked the stimulatory of insulin on MAPK activation and cell growth as wortmannin blocked the stimulatory of insulin on MAPK activation and mitogenesis (20). Thus, it appears from the current study as well as from our previous results that insulin-mediated PI3-kinase activation leads to activation of distinct signal transduction pathways that mediate MAPK activation and cell growth as well as MKP-1 induction to terminate MAPK signaling.

Several studies using NIH3T3 fibroblasts and other cell types indicate that MKP-1 induction may be mediated by SAPK signaling and/or ERK signaling pathways (34–35). Our preliminary studies with anisomycin, a potent stimulator of JNK/SAPK, indicate that MKP-1 mRNA expression can be induced by anisomycin in VSMCs with a resultant inhibition of MKP-1 mRNA expression. The above findings suggested the existence of a complex, cell type-specific cross-talk between the ERKs/SAPK and p38 MAPK signaling cascades. Alternatively, MAPK signaling pathways may be interacting with the NO pathway to cause MKP-1 induction. To test this possibility, VSMCs were exposed to PD98059 and SB203580, respectively, followed by insulin treatment and examined for iNOS induction and MKP-1 expression. Inhibition of ERKs with PD98059 completely blocked the effects of insulin on iNOS induction and MKP-1 mRNA expression.

FIG. 7. Cross-talk between MAPK and NOS signaling pathways. Effect of inhibition of MAPK signaling on insulin-induced iNOS protein (A) and MKP-1 expression (B). Duplicate dishes of VSMCs were pretreated with vehicle alone, PD98059 (50 μM), and SB203580 (0.3 μM) for 30 min followed by insulin (100 nm) for 30 min. Equal amounts of proteins (50 μg) were examined for iNOS expression by Western blot analysis (see A). In another set of experiments, RNA was extracted from control, insulin, and inhibitor + insulin-treated cells followed by Northern blot analyses of RNA for MKP-1 mRNA expression (see B). A representative autoradiogram is shown. Similar results were obtained in three different experiments. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

In contrast to WKY, VSMCs from SHR exhibit a marked insulin resistance in terms of MKP-1 induction. The observed impairment in insulin-induced MKP-1 mRNA expression may be responsible for the sustained MAPK activation and increased cell growth observed by us in VSMCs isolated from SHR (see Ref. 20). To our knowledge, this is the first study to demonstrate insulin-mediated expression of MKP-1 mRNA in VSMCs and its abnormal induction in hypertension. Other studies have demonstrated induction of MKP-1 by FBS, AII, and platelet-derived growth factor (13). Studies by Lai et al. (37) reported that balloon injury of rat carotid artery was accompanied by a marked increase in MKP-1 mRNA expression, whereas p44 MAPK activity was increased. The results presented in this study add a new dimension to the observations that sustained MAPK activation seen in hypertension and atherosclerosis may be due to inherent reductions in MKP-1 mRNA expression resulting from defective regulation of MKP-1 gene expression in response to insulin and IGF-1. These observations led us to examine the precise intracellular insulin signal transduction pathway(s) that mediate MKP-1 expression.

Given the knowledge that insulin acts as a potent vasodilator and increases the production of NO in endothelial cells (38), we examined the contribution of the NO signaling pathway in insulin-mediated MKP-1 expression in VSMCs. In the initial studies, we observed that incubation of VSMCs with low concentrations of SNP, an exogenous NO donor, rapidly induced MKP-1 mRNA expression. The effects of SNP on MKP-1 expression were additive. Furthermore, inhibition of NOS signaling by treatment of VSMCs with l-NMMA, a potent NOS inhibitor, completely blocked insulin-induced MKP-1 expression. In addition, blockade of cGMP, a downstream effector of NOS signaling by treatment with Rp-cGMP, a cGMP antagonist, prevented insulin-induced MKP-1 expression. These preliminary results suggest that NOS signaling pathway may play a significant role in insulin-mediated MKP-1 expression. Further confirmation of the role of NOS/cGMP signaling in insulin-mediated MKP-1 expression came from studies demonstrating a rapid induction of iNOS protein by insulin. The insulin effect on iNOS was accompanied by a rapid increase in cellular cGMP levels. Pretreatment of VSMCs with the NOS inhibitor, l-NMMA, completely blocked cGMP production and MKP-1 expression. Treatment of VSMCs with 8-bromo-cGMP, a cGMP agonist, also mimicked the effect of insulin on MKP-1 expression and bypassed the inhibitory effects of l-NMMA. The effects of cGMP and insulin on MKP-1 expression were not synergistic. These results clearly indicate that the NOS/cGMP signaling pathway may play a dominant role in insulin-mediated MKP-1 expression. In support of our observations, studies by Sugimoto et al. (39) indicate that atrial natriuretic peptide, a potent vasorelaxing factor, inhibits MAPK activation and cell proliferation by inducing MKP-1 via activation of guanylate cyclase.

The inducible form of NOS is the predominant isoform of the NOS family of proteins that is expressed in VSMCs (40), although recent studies suggest that VSMCs may express constitutive endothelial NOS as well (40). In the present study we observed that induction of iNOS protein was rapid upon treatment with insulin and the protein levels return to basal values in 1 h, suggesting a rapid turnover.

To understand further the mechanism whereby insulin regulates NOS/cGMP signaling in VSMCs, we examined the contribution of the PI3-kinase pathway in insulin-mediated iNOS induction. Insulin caused a rapid time- and dose-dependent increase in IRS-1-associated PI3-kinase activity. Treatment with wortmannin, a potent PI3-kinase inhibitor, completely blocked PI3-kinase activation by insulin and inhibited the induction of iNOS, cGMP production, and MKP-1 mRNA expression. Studies by Zheng and Quon (38) in human vascular endothelial cells suggested that PI3-kinase may participate in the vasodilatory effects of insulin by increasing the production of NO. In addition to MKP-1 induction, PI3-kinase activation is required for MAPK activation and cell growth as wortmannin blocked the stimulatory of insulin on MAPK activation and mitogenesis (20). Thus, it appears from the current study as well as from our previous results that insulin-mediated PI3-kinase activation leads to activation of distinct signal transduction pathways that mediate MAPK activation and cell growth as well as MKP-1 induction to terminate MAPK signaling.

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expression. In contrast to PD98059, inhibition of p38\textsuperscript{HOG} kinase activity with SB203580 did not affect iNOS induction by insulin but prevented MKP-1 mRNA expression. Given that insulin increases only ERKs activity in VSMCs and does not affect p38\textsuperscript{HOG} kinase activity, it appears that ERK signaling pathways may cross-talk with iNOS signaling pathway to regulate MKP-1 induction in response to insulin.

Several recent studies suggest that p38 MAPK activity increases upon the withdrawal of serum, and the addition of growth factors as well as insulin inhibit p38 MAPK activity (41). The presence of detectable levels of phosphorylated p38 MAPK in VSMCs under basal conditions suggests that this enzyme may be needed to maintain adequate expression of MKP-1 mRNA in the basal state. Upon insulin treatment VSMCs may use the MAPK-mediated NOS signaling pathway to cause MKP-1 expression. Further studies with constitutively active as well as dominant negative mutants of ERK and p38 MAPK will help in understanding the exact contribution of ERKs and p38 in NOS activation and MKP-1 induction. It should be noted that the NOS signaling pathway does not directly control MAPK activation in VSMCs, as inhibition of NOS with l-NMMA did not prevent ERK activation but increased its activation status presumably due to inhibition of MKP-1 expression.

In VSMCs from SHR, it appears that defective MKP-1 induction by insulin may be due to reductions in PI3-kinase-generated signals leading to impaired induction of iNOS and reduced cGMP production. The above defects in MKP-1 induction could be corrected by direct treatment with SNP, a NO donor, as well as with cGMP agonist.

In summary, the results of the present study indicate that insulin induces MKP-1 expression in VSMCs by employing PI3-kinase-initiated signals leading to the induction/activation of iNOS resulting in cGMP production that may mediate MKP-1 gene expression. The observed impairment in MKP-1 induction in SHR is due to defective NOS signaling leading to sustained MAPK activation and accelerated cell growth. This study also highlights the possibility of potential cross-talk between MAPK and NOS signaling pathways.

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