Role of the actin cytoskeleton in angiotensin II signaling in human vascular smooth muscle cells[1]

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Abstract: Angiotensin II (Ang II) regulates vascular smooth muscle cell (VSMC) function by activating signaling cascades that promote vasoconstriction, growth, and inflammation. Subcellular mechanisms coordinating these processes are unclear. In the present study, we questioned the role of the actin cytoskeleton in Ang II mediated signaling through mitogen-activated protein (MAP) kinases and reactive oxygen species (ROS) in VSMCs. Human VSMCs were studied. Cells were exposed to Ang II (10^{-7} mol/L) in the absence and presence of cytochalasin B (10^{-6} mol/L, 60 min), which disrupts the actin cytoskeleton. Phosphorylation of p38MAP kinase, JNK, and ERK1/2 was assessed by immunoblotting. ROS generation was measured using the fluoroprobe chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (4 μmol/L). Interaction between the cytoskeleton and NADPH oxidase was determined by evaluating the presence of p47phox in the Triton X-100 insoluble membrane fraction. Ang II significantly increased phosphorylation of p38MAP kinase, JNK, and ERK1/2 (two- to threefold above control, $p < 0.05$). Cytochalasin B pretreatment attenuated p38MAP kinase and JNK effects ($p < 0.05$) without altering ERK1/2 phosphorylation. ROS formation, which was increased in Ang II stimulated cells, was significantly reduced by cytochalasin B ($p < 0.01$). p47phox, critically involved in NADPH oxidase activation, colocalized with the actin cytoskeleton in Ang II stimulated cells. Our data demonstrate that Ang II mediated ROS formation and activation of p38MAP kinase and JNK, but not ERK1/2, involves the actin cytoskeleton in VSMCs. In addition, Ang II promotes interaction between actin and p47phox. These data indicate that the cytoskeleton is involved in differential MAP kinase signaling and ROS generation by Ang II in VSMCs. Together, these studies suggest that the cytoskeleton may be a central point of crosstalk in growth- and redox-signaling pathways by Ang II, which may be important in the regulation of VSMC function.

Key words: superoxide, NADPH oxidase, p38MAP kinase, JNK, ERK1/2.

Résumé : L'angiotensine II (Ang II) régule la fonction des cellules musculaires lisses vasculaires (CMLV) en activant des cascades de signalisation qui favorisent la vasoconstriction, la croissance et l'inflammation. Les mécanismes sous-cellulaires coordonnant ces processus sont obscurs. Dans la présente étude, nous avons remis en question le rôle du cytosquelette d'actine dans la signalisation par l'Ang II des protéines kinases activées par un mitogène (MAP) et des espèces oxygénées radicalaires (ROS) dans les CMLV. Nous avons exposé des CMLV humaines à l'Ang II (10^{-7} mol/L) en absence et en présence de cytochalasine B (10^{-6} mol/L, 60 min), qui désorganise le cytosquelette d'actine. Nous avons évalué la phosphorylation de la MAP kinase p38, de JNK et de ERK1/2 par immunobuvardage. Nous avons mesuré la production des ROS à l'aide du marqueur fluorescent chlorométhyl-2′,7′-dichloro-dihydro-fluorescéine diacétate (4 μmol/L). Nous avons déterminé l'interaction entre le cytosquelette et la NADPH oxydase en évaluant la présence de p47phox dans la fraction membranaire insoluble du Triton X-100. L'Ang II a augmenté de manière significative la phosphorylation de la MAP kinase p38, JNK et ERK1/2 (facteur 2–3 supérieur aux valeurs témoins, $p < 0{,}05$). Un prétraitement avec la cytochalasine B a atténué les effets de la MAP kinase p38 et de JNK ($p < 0{,}05$) sans modifier la phosphorylation de ERK1/2. La cytochalasine B a réduit significativement ($p < 0{,}01$) la formation des ROS dans les cellules stimulées par l'Ang II. La protéine p47phox, impliquée étroitement dans l'activation de la NADPH oxydase, a été co-localisée avec le cytosquelette d'actine dans les cellules stimulées par l'Ang II. Nos résultats démontrent que la formation des ROS et l'activation de la MAP kinase p38 et de JNK, mais pas de ERK1/2, par l'Ang II impliquent la présence du cytosquelette d'actine dans les CMLV. De plus, l'Ang II favorise l'interaction entre l'actine et p47phox. Ces résultats indiquent que le cytosquelette est impliqué dans la signalisation des MAP kinases et la production des ROS par l'Ang II dans les CMLV. Ces études laissent croire que le cytosquelette pourrait être un point central de couplage dans les voies de signalisation redox et de croissance par l'Ang II, ce qui pourrait être important dans la régulation de la fonction des CMLV.

Mots clés : superoxyde, NADPH oxydase, Map kinase p38, JNK, ERK1/2.

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Introduction

Angiotensin II (Ang II), a key regulator of vascular smooth muscle cell (VSMC) function, has pleiotropic actions in the vasculature. Ang II stimulates vasoconstriction, induces VSMC growth, is fibrogenic, promotes migration, and is pro-inflammatory (Touyz and Schiffrin 2000; Frank and Eguchi 2003). These processes are associated with activation of complex signaling pathways and dynamic remodeling of the actin cytoskeleton (Touyz and Schiffrin 2000; Frank and Eguchi 2003; Wesselman and De Mey 2002). Mechanisms implicated in Ang II induced regulation of actin filament formation include heat shock protein 27, p38MAP kinase, and reactive oxygen species (ROS) (Meloche et al. 2000; Pichon et al. 2004; Gerhoffer and Gunst 2001). In addition, phosphorylation of cytoskeleton-associated tyrosine kinases, such as c-Src, p130Cas, paxillin, and focal adhesion kinase (FAK), are involved in actin cytoskeleton rearrangement following Ang II stimulation (Ishida et al. 1999; Kyaw et al. 2004; Takahashi and Berk 1996).

In addition to maintaining cellular structural integrity through its three-dimensional network of filamentous polymers, the cytoskeleton may participate in agonist-stimulated signal transduction (Wesselman and De Mey 2002). Actin disorganization reduced Ang II induced Ca^{2+} release from intracellular stores and Ca^{2+} influx in cultured rat aortic smooth muscle cells (Samain et al. 1999). Disruption of the actin filament network by cytochalasin B decreased Ang II mediated vascular contraction and ERK1/2 activity (Matrougui et al. 2001). An intact cytoskeleton is essential for NADPH oxidase activation and ROS generation and plays a major role in TNFα signaling in neutrophils (Gu et al. 2002). On the other hand, in epithelial cells, actin disruption by cytochalasin D was associated with increased NF-κB activation and augmented IL-8 production (Nemeth et al. 2004). Taken together, these data suggest that the actin cytoskeleton is closely in agonist-mediated signal transduction.

Ang II stimulates contraction, growth, and inflammation by activating multiple members of the mitogen-activated protein (MAP) kinase family, including ERK1/2, p38MAP kinase, and JNK (Touyz et al. 1999, 2003*a*; Ishihata et al. 2002). In addition, generation of ROS by NADPH oxidase, a complex enzyme comprising p22phox, gp91phox, p47phox, and p67phox subunits, has been shown to be important in Ang II mediated redox signal transduction (Seshiah et al. 2002; Touyz et al. 2002; Lassegue and Clempus2003). Since these signaling molecules are involved in processes associated with cytoskeletal remodeling, we questioned whether the actin cytoskeleton is an upstream regulator of MAP kinases and ROS production in VSMCs stimulated by Ang II. Using cytochalasin B, which caps the barbed end of actin filaments promoting depolymerization of filamentous actin, and therefore disruption of the cytoskeleton, we demonstrate that Ang II mediated ROS formation and activation of p38MAP kinase and JNK, but not ERK1/2, require an intact actin cytoskeleton. This interaction between cytoskeletal dynamics and Ang II induced signaling may be an important point of crosstalk in activated VSMCs.

Materials and methods

Sources of reagents

Cytochalasin B was purchased from Sigma Chemical Co. (St. Louis, Missouri). 5-(and 6)-Chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CMH_2-DCFDA) was from Molecular Probes, Inc. (Eugene, Oregon). Monoclonal p47phox antibodies were kindly provided by Dr. M.T. Quinn (Montana State University, Bozman, Mont.).

Cell culture

The study was approved by the Ethics Committee of the Clinical Research Institute of Montreal. Healthy volunteers (30–65 years) were recruited at the Clinical Research Institute of Montreal Hypertension Clinic. Biopsies of gluteal subcutaneous tissue were obtained under local anesthetic and vessels dissected as previously described (Touyz et al. 2002, 2003*b*; Schiffrin et al. 1994). VSMCs were isolated, cultured, and characterized as previously detailed (Touyz et al. 2002, 2003*b*). At subconfluence, cells were placed in quiescent serum-free culture medium for 24 h prior to experimentation. Low-passaged cells (passages 2–7) were used for experiments.

Measurement of ROS in intact cells

Intracellular H_2O_2 levels were measured with the fluoroprobe CMH_2-DCFDA (Touyz et al. 2003*b*; Touyz and Schiffrin 2001) in unstimulated cells and in cells exposed to Ang II (10^{-8}–10^{-6} mol/L) in the absence and presence of 10^{-6} mol cytochalasin B/L (60-min pre-incubation). Cells, which were used for single experiments, were washed in modified Hanks buffered saline containing (millimoles per litre) 137 NaCl, 4.2 $NaHCO_3$, 3 $NaHPO_4$, 5.4 KCl, 0.4 KH_2PO_4, 1.3 $CaCl_2$, 0.5 $MgCl_2$, 0.8 $MgSO_4$, 10 glucose, and 5 HEPES (pH, 7.4) and loaded with tempo-9-AC or CMH_2-DCFDA (4 μmol/L), dissolved in dimethyl sulfoxide, and incubated for 30 min at room temperature.

Fluorescence was measured by fluorescence digital imaging using an Axiovert 135 inverted microscope (40× oil immersion objective) and Attofluor digital fluorescence system (Zeiss, Germany) using excitatory wavelengths of 360 and 495 nm for tempo-9AC and CMH_2-DCFDA, respectively, as previously described (Touyz et al. 2002, 2003*b*; Schiffrin et al. 1994). Video images of fluorescence at 520 nm emission were obtained using an intensified CCD camera system (Zeiss) with the output digitized to a resolution of 512 × 480 pixels. Effects of Ang II were evaluated in single cells in cell clusters. Fifteen to 25 cells were examined per field. Data are expressed as the Ang II induced change in fluorescence.

Isolation of cytoskeleton

Following stimulation, cells were pelleted and resuspended at 10×10^9 cells/100 μL lysis buffer (100 mmol Tris–HCl/L, pH 7.4, 1% Triton X-100) containing 1 mmol EGTA/L and protease inhibitors (0.25 mmol leupeptin/L, 1 mmol diisopropyl fluorophosphate/L) (El Benna et al. 1999). Cells were sonicated for 7 s and maintained on ice for 10 min. Cell sonicates were loaded on 300 μL of 6% sucrose containing 1% Triton X-100 and 100 mmol Tris/L, pH 7.4, and centrifuged at 72 000 rpm (Beckman TLA 100.2

rotor) at 4 °C for 38 min. The upper 100-µL fraction, the Triton-soluble portion, was separated from the pellet, the Triton-insoluble cytoskeleton fraction, and loaded onto SDS–PAGE.

Immunoblotting

After SDS–PAGE separation of proteins, samples were transferred to PVDF membranes. Membranes, blocked in TBS-Tween containing 5% nonfat dry milk, were incubated with monoclonal p47phox antibody (1:750). Membranes were washed three times with TBS-Tween and incubated with horseradish peroxidase conjugated second antibody (1:6000) (1.5 h, 24 °C). Immunoreactive proteins were detected by chemiluminescence. Blots were analyzed densitometrically (Image-Quant software; Molecular Dynamics, Sunnyvale, Calif.).

To evaluate whether cytoskeletal disruption influences MAP kinase signaling by Ang II, effects of Ang II (10^{-7} mol/L, 5–15 min) on MAP kinase phosphorylation were determined in the absence and presence of cytochalasin B (10^{-6} mol/L, 60-min pretreatment). Phosphorylation of p38MAP kinase, JNK, and ERK1/2 was assessed by immunoblotting using phospho-specific antibodies to p38MAP kinase, JNK, and ERK1/2 (1:1000) (Cell Signaling Technology Inc., Beverly, Mass.). Total protein MAP kinase content was determined by immunoblotting using anti-p38MAP kinase, anti-JNK, and anti-ERK1/2 antibodies (1:1000) (Cell Signaling Technology Inc.) as previously described (Touyz et al. 2004).

Analysis

Data obtained from digital imaging studies, where multiple cells were examined in each experimental field, were calculated as the mean response per experiment and then as the mean of multiple experiments. Each experiment was performed at least three times. Values are presented as means ± SEM. Data were analyzed by ANOVA or Student's *t* test. Tukey–Kramer's correction was used to compensate for multiple testing procedures. A p value of <0.05 was significant.

Results

Cytochalasin B differentially regulates p38MAP kinase, JNK, and ERK1/2 by Ang II

To evaluate whether the cytoskeleton plays a functional role in MAP kinase signaling by Ang II, we assessed effects of cytochalasin B, which disrupts F-actin assembly, on Ang II induced phosphorylation of p38MAP kinase, JNK, and ERK1/2. In the present study, Ang II was used at a dose of 10^{-7} mol/L, which we previously demonstrated to induce maximal responses (Touyz et al. 2004).

As demonstrated in Figs. 1–3, Ang II stimulation significantly increased phosphorylation of all three MAP kinases in VSMCs ($p < 0.01$). Responses were rapid, with maximal actions obtained within 5 min of stimulation. Cytochalasin B alone had no effect on basal MAP kinase activation but significantly attenuated Ang II mediated phosphorylation of p38MAP kinase and JNK ($p < 0.05$) (Figs. 1 and 2). Inhibitory effects were more pronounced in cells that were exposed to Ang II for longer time periods (5 vs. 15 min). Unlike p38MAP kinase and JNK, ERK1/2 activation was not significantly modified by cytochalasin B (Fig. 3). Nei-

Fig. 1. Immunoblot detection of Ang II induced phosphorylation of p38MAP kinase in the absence and presence of cytochalasin B (Cyt) (10^{-6} mol/L, 1 h). VSMCs were stimulated with Ang II (10^{-7} mol/L) for 5–15 min. Protein was extracted and 15 µg of protein loaded per lane. Blots were probed for phosphorylated and total p38MAP kinase using phospho-specific and p38MAP kinase specific antibodies (1:1000). Results are expressed as a percentage of control (Con) (taken as 100%). Results are means ± SEM of three experiments. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. control; $^{+}p < 0.05$ vs. cytochalasin; $^{\dagger}p < 0.05$; $^{\ddagger}p < 0.01$.

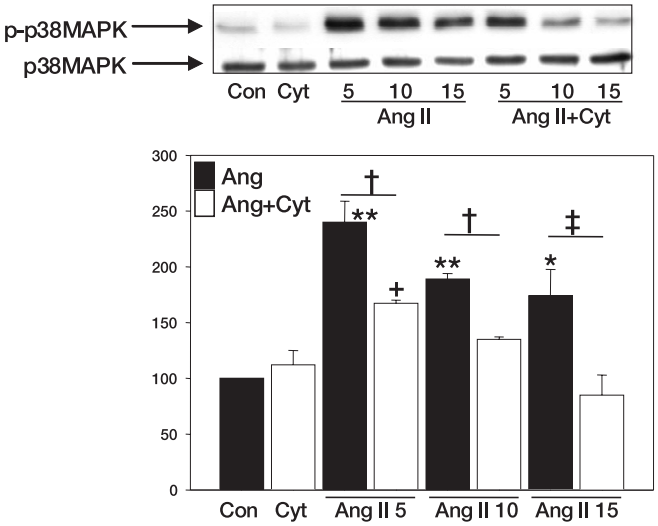


Fig. 2. Immunoblot detection of Ang II induced phosphorylation of JNK in the absence and presence of cytochalasin B (Cyt) (10^{-6} mol/L, 1 h). VSMCs were stimulated with Ang II (10^{-7} mol/L) for 5–15 min. Protein was extracted and 15 µg of protein loaded per lane. Blots were probed for phosphorylated and total JNK using phospho-specific and JNK-specific antibodies (1:1000). Results are expressed as a percentage of control (Con) (taken as 100%). Results are means ± SEM of three experiments. $^{**}p < 0.01$ vs. control; $^{+}p < 0.05$ and $^{++}p < 0.01$ vs. cytochalasin; $^{\ddagger}p < 0.01$.

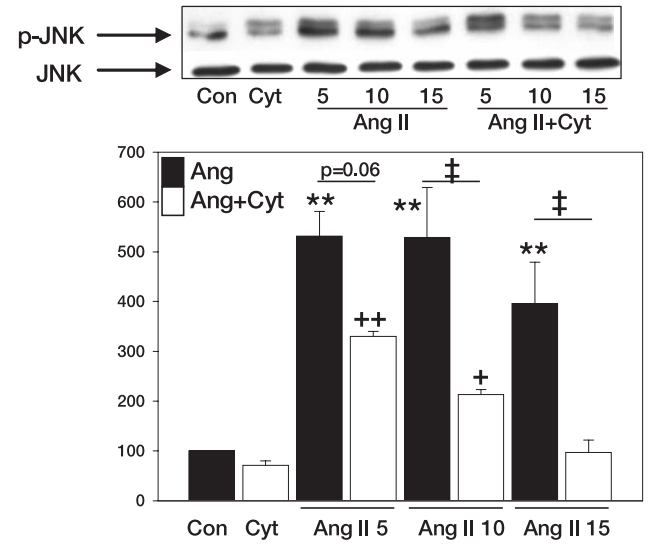
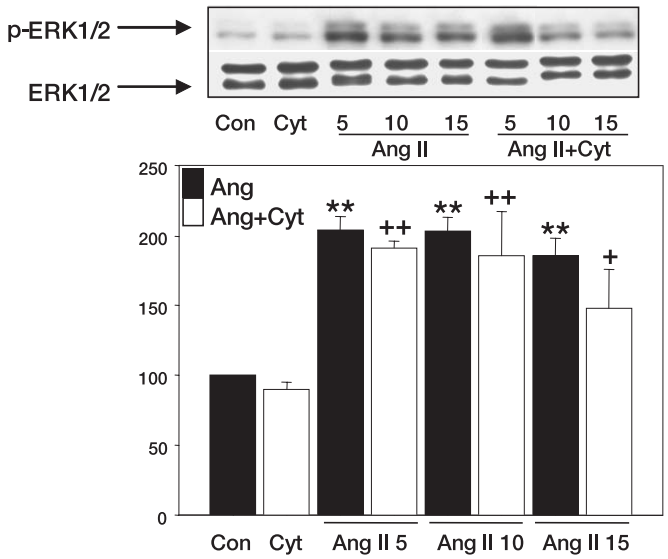


Fig. 3. Immunoblot detection of Ang II induced phosphorylation of ERK1/2 in the absence and presence of cytochalasin B (Cyt) (10^{-6} mol/L, 1 h). VSMCs were stimulated with Ang II (10^{-7} mol/L) for 5–15 min. Protein was extracted and 15 µg of protein loaded per lane. Blots were probed for phosphorylated and total ERK1/2 using phospho-specific and ERK1/2-specific antibodies (1:1000). Results are expressed as a percentage of control (Con) (taken as 100%). Results are means ± SEM of three experiments. $^{**}p < 0.01$ vs. control; $^{+}p < 0.05$ and $^{++}p < 0.01$ vs. cytochalasin.



ther cytochalasin B nor Ang II influenced total protein content of p38MAP kinase, JNK, and ERK1/2.

Cytochalasin B attenuates Ang II induced generation of ROS

Ang II induced a slow and sustained increase in ROS formation, as evidenced by increased CMH_2-DCFDA fluorescence. Maximal responses were obtained within 15–20 min. As previously demonstrated, catalase abrogated CMH_2-DCFDA fluorescence, indicating that the fluorescence signal derives predominantly from H_2O_2 (Touyz et al. 2002). Ang II dose-dependently increased H_2O_2 formation (Fig. 4). Preexposure of VSMCs to cytochalasin B significantly reduced, but did not abolish, Ang II mediated ROS generation.

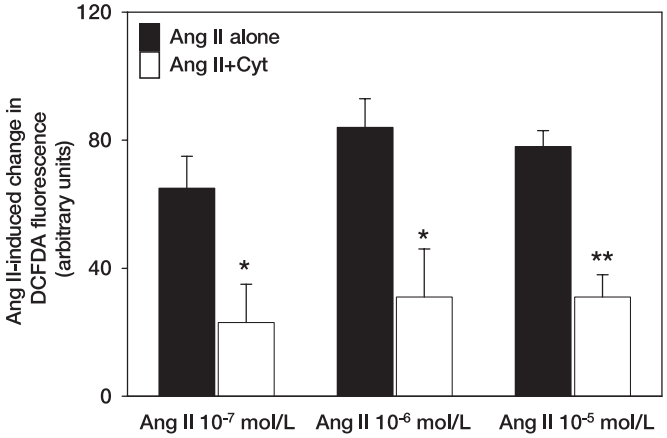
NAD(P)H oxidase association with the cytoskeleton in Ang II stimulated VSMCs

To determine whether NAD(P)H oxidase interacts with the cytoskeleton in Ang II stimulated VSMCs, we assessed the association between the actin cytoskeleton and p47phox, an NAD(P)H oxidase subunit critically involved in activation of the enzyme. As demonstrated in Fig. 5, p47phox is weakly expressed in the Triton-insoluble cytoskeleton-rich fraction from control cells. Stimulation of VSMCs with Ang II resulted in a marked increase in p47phox abundance in the Triton-insoluble fraction, suggesting that Ang II promotes association between p47phox and the actin cytoskeleton.

Discussion

Major findings from the present study demonstrate that

Fig. 4. Bar graphs demonstrating the effects of Ang II on CMH_2-DCFDA fluorescence in the absence and presence of cytochalasin B. Cells were pre-exposed to cytochalasin B (Cyt) (10^{-6} mol/L) for 1 h prior to addition of Ang II. Data are means ± SEM of three experiments with each experimental field comprising 10–20 cells. $^{*}p < 0.05$ and ** $p < 0.01$ vs. Ang II counterpart.



disruption of the actin cytoskeleton with cytochalasin B results in inhibition of Ang II mediated activation of p38MAP kinase and JNK, but not of ERK1/2, and attenuation of intracellular H_2O_2 production. Furthermore, we show that Ang II promotes association between the actin cytoskeleton and p47phox, a major regulatory subunit of VSMC NAD(P)H oxidase (Lassegue and Clempus 2003; Touyz et al. 2003*b*; Li et al. 2002). Our data indicate that the cytoskeleton is involved in differential MAP kinase signaling and ROS generation by Ang II in VSMCs. These studies suggest that the cytoskeleton may be a central point of crosstalk in growth- and redox-signaling pathways by Ang II, which may be important in coordinated regulation of VSMC function.

Ang II is a multifunctional peptide that mediates a plethora of actions in VSMCs. Ang II induces contraction, cell growth, migration, and differentiation and is profibrotic (Touyz and Schiffrin 2000; Frank and Eguchi 2003; Wesselman and De Mey 2002). In addition, compelling evidence indicates that Ang II has important pro-inflammatory properties in the vascular wall, stimulating generation of ROS and production of inflammatory cytokines and adhesion molecules through the cytoplasmic transcription factor NF-κB and activation of MAP kinases (Touyz and Schiffrin 2000; Frank and Eguchi 2003; Suzuki et al. 2003). Many of these processes involve reorganization of the cytoskeleton and subsequent signaling to the contractile and growth-promoting molecular machinery. Ang II stimulation induces F-actin association with cytoskeletal structures, mediated in part through phosphorylation of nonreceptor tyrosine kinases, such as paxillin and FAK (Ishida et al. 1999; Kyaw et al. 2004; Takahashi and Berk 1996). Emerging evidence indicates that in addition to the structural importance in maintaining VSMC integrity, the actin cytoskeleton plays a major role in regulating downstream signaling events by vasoactive agents (Wesselman and De Mey 2002). Cytoskeletal disruption with cytochalasin D prevented ET-1-stimulated FAK-

Fig. 5. p47phox is associated with the cytoskeleton. p47phox is recovered from the Triton X insoluble fraction (cytoskeleton). VSMCs were stimulated with vehicle (water) (control) or Ang II (10^{-7} mol/L, 10 min) and then extracted with Triton X-100 into insoluble (cytyoskeleton) and soluble fractions. A 48- to 50-kDa protein immunoreactive with antibodies to p47phox was recovered primarily in the detergent-insoluble fraction, with enhanced effects in Ang II stimulated cells. α-Actin was used as a housekeeping protein to confirm equal loading, as demonstrated in the lower panel. Membranes were stripped and reprobed with anti-α-actin antibody. Immunoblots are representative of three experiments. PC, positive control; VSMC, lysate.

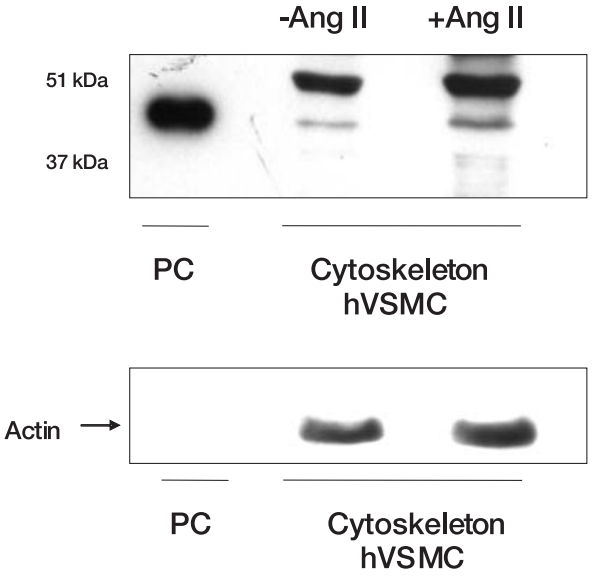
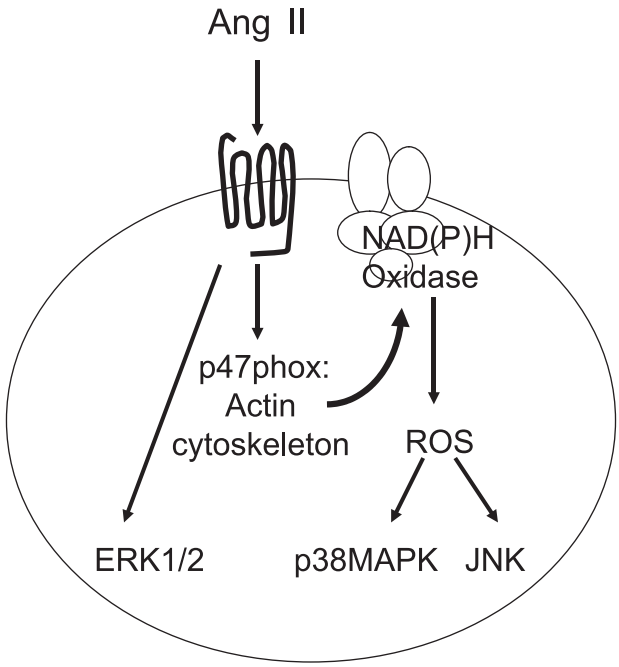


Fig. 6. Hypothetical scheme whereby the actin cytoskeleton regulates redox-sensitive MAP kinase signaling by Ang II. Ang II stimulation of VSMCs results in cytoskeletal remodeling and interaction between p47phox and the actin cytoskeleton. The p47phox–cytoskeletal interaction promotes translocation of cytosolic NAD(P)H oxidase subunits with consequent activation of VSMC NAD(P)H oxidase and generation of ROS. Increased intracellular ROS activate redox-sensitive signaling molecules, including p38MAP kinase and JNK, but not ERK1/2.



regulated p130Cas/Crk/Pyk2/c-Src-mediated JNK activation in cardiomyocytes (Kodama et al. 2003). In rabbit aortic strips, cytochalasin B blocked norepinephrine-induced contraction (Dresel and Knickle 1987), and in rat aortic VSMCs, cytochalasin D reduced Ang II induced FAK phosphorylation and decreased ERK1/2-mediated protein synthesis (Govindarajan et al. 2000).

In the present study, we provide the novel finding that the actin filament network differentially regulates MAP kinase signaling by Ang II. Ang II mediated activation of p38MAP kinase and JNK, but not ERK1/2, was reduced by cytochalasin B. Thus, whereas p38MAP kinase and JNK require an intact actin cytoskeleton for full activation by Ang II, ERK1/2 does not. Matrougui et al. (2001) reported that cytochalasin B markedly attenuates pressure-induced ERK1/2 activation but only modestly reduces Ang II stimulated ERK1/2 phosphorylation in mesenteric resistance arteries, suggesting that the cytoskeleton may be more important in pressure-mediated than Ang II induced ERK1/2 activation. Differential association between VSMC contractile machinery and MAP kinases was suggested in VSMCs cultured in three-dimensional matrix, where p38MAP kinase was found to be more important than ERK1/2 with respect to VSMC-induced matrix contraction (Li et al. 2003). c-Src, Pyk2, FAK, and paxillin are actin cytoskeletal associated proteins that have been implicated to regulate MAP kinases in VSMCs (Ishida et al. 1999; Kyaw et al. 2004; Takahashi and Berk 1996).

In addition to modulating activation of MAP kinases, the actin cytoskeleton may influence redox signaling by Ang II. To address this, we measured H_2O_2 production in the presence of cytochalasin B. Although disruption of the actin cytoskeleton with cytochalasin B did not abrogate Ang II induced H_2O_2 formation, responses were markedly attenuated, suggesting that an intact cytoskeleton is important, but not critical, for ROS production by Ang II. These findings confirm and extend those for neutrophils and endothelial cells (El Bekay et al. 2003; Morimatsu et al. 1997; Grogan et al. 1997; Wientjes et al. 2001; Clements et al. 2003). Processes whereby the cytoskeleton influences Ang II induced ROS formation probably involve regulation of NAD(P)H oxidase, the major enzymatic source of vascular ROS (Lassegue and Clempus 2003). In activated neutrophils, p47phox, p67phox, and p40 phox associate with F-actin (Gu et al. 2003; Wu et al. 2003). In endothelial cells, p47phox localizes with the cytoskeleton and participates in VEGF and TNFα signaling (Gu et al. 2002, 2003; Wu et al. 2003). Here, we demonstrate that p47phox, an essential NAD(P)H oxidase regulatory subunit, colocalizes with the actin cytoskeleton and that in Ang II stimulated cells, the p47phox–cytoskeleton interaction is increased. These data suggest that the actin filament network, by promoting p47phox regulation of NAD(P)H oxidase, is involved in Ang II induced ROS formation and redox signaling in VSMCs.

Previous studies from our laboratory and others demonstrated that Ang II regulates p38MAP kinase and JNK, but

not ERK1/2, through redox-sensitive signaling cascades in VSMCs (Touyz et al. 2004; Viedt et al. 2000). The reasons for this differential regulation remain unclear, but it is possible that activation of the actin cytoskeleton may be important. This is reinforced in the present study by the finding that cytochalasin B attenuated formation of ROS and decreased activation of p38MAP kinase and JNK without influencing ERK1/2. We propose that Ang II induces p47phox–actin association, which promotes site-directed activation of NAD(P)H oxidase to generate ROS. ROS in turn regulate activation of p38MAP kinase and JNK (Fig. 6). ERK1/2 phosphorylation by Ang II appears to be independent of the actin cytoskeleton and does not rely on ROS for its activation (Touyz et al. 2004; Viedt et al. 2000).

In conclusion, the actin cytoskeleton plays an important regulatory role in Ang II signaling in VSMCs. The actin cytoskeleton may couple NAD(P)H oxidase-mediated generation of ROS to redox-sensitive MAP kinases, such as p38MAP kinase and JNK, and may act as a point of crosstalk between multiple signaling pathways activated by Ang II in VSMCs.

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