Angiotensin II Stimulates p21-Activated Kinase in Vascular Smooth Muscle Cells
Role in Activation of JNK

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Abstract—Angiotensin II (Ang II) has been previously shown to stimulate the extracellular signal–regulated kinase (ERK)1/2 and c-Jun N-terminal kinase (JNK) mitogen-activated protein (MAP) kinase family members. Little is known regarding the upstream signaling molecules involved in Ang II–mediated JNK activation. Ang II has been shown to activate the Janus kinase/signal transducer(s) and activator(s) of transcription (JAK/STAT) pathway, suggesting similarities to cytokine signaling. In response to cytokines such as interleukin-1 and tumor necrosis factor–α, the p21-activated kinase (PAK) has been identified as an upstream component in JNK activation. Therefore, we hypothesized that PAK may be involved in JNK activation by Ang II in vascular smooth muscle cells (VSMCs). αPAK activity was measured by myelin basic protein phosphorylation in rat aortic VSMCs. In response to Ang II, αPAK was rapidly stimulated within 1 minute, with a peak (5-fold increase) at 30 minutes. αPAK stimulation preceded activation of JNK in VSMCs. Ang II–mediated activation of both αPAK and JNK was Ca2+ dependent and inhibited by downregulation of phorbol ester–sensitive protein kinase C isoforms (by pretreatment with phorbol 12,13-dibutyrate) but not by pretreatment with GF109203X. Activation of both PAK and JNK was partially inhibited by tyrosine kinase inhibitors but not by specific Src inhibitors, suggesting regulation by a tyrosine kinase other than c-Src. Finally, introduction of dominant negative PAK markedly reduced the JNK activation by Ang II in both Chinese hamster ovary and COS cells stably expressing the Ang II type 1 receptor (AT1R). Our data provide evidence for αPAK as an upstream mediator of JNK in Ang II signaling and extend the role of Ang II as a proinflammatory mediator for VSMCs. (Circ Res. 1998;82:1272-1278.)

Key Words: angiotensin II ■ mitogen-activated protein kinase ■ vascular smooth muscle cell ■ c-Jun kinase

The renin-angiotensin system has been implicated in several cardiovascular diseases, including hypertension, restenosis after balloon injury, atherosclerosis, and myocardial infarction. Ang II is the main effector of the renin-angiotensin system and has previously been shown to promote growth and inhibit apoptosis,1–4 thus contributing to cardiac and vascular hypertrophy. The growth-promoting and antiapoptotic effects of Ang II depend on the regulation of transcription factors, which induce the expression of specific genes leading to protein synthesis, cell division, or inhibition of cell death pathways. Many of the signaling events relevant for these complex processes are mediated through activation of the MAP kinase family members ERK1/2 and JNK, which have been shown to be activated by Ang II.5,6 The MAP kinases activate transcription factors involved in the regulation of cell proliferation and differentiation as well as apoptosis, including c-Jun, Elk-1, and ATF2.7 Activation of ERK1/2 and JNK by Ang II is mediated by the AT1R.8 The AT1R has been recently cloned and shown to belong to the seven transmembrane–spanning, G protein–coupled receptor family.9,10 The signal transduction events that link AT1R activation to the stimulation of ERK1/2 have been elucidated in recent years11; however, the mechanisms leading to JNK activation are less well defined.6

The JNK/stress-activated protein kinase members of the MAP kinase family have been shown to be stimulated by both inflammatory stimuli (eg, interleukin-1 and TNF-α) and cellular stresses (eg, anisomycin, hyperosmolarity, and UV light).12 The small G proteins Rac and Cdc42Hs are important upstream mediators of JNK activation because constitutively active mutants of these small GTPases enhance JNK activity, whereas dominant negative mutants block activation of JNK.13,14 A putative downstream component of Rac and Cdc42Hs in the signaling pathway leading to JNK activation is PAK,15 which becomes activated on binding to GTP-bound Rac or Cdc42Hs. PAK has been implicated in the regulation

Received July 16, 1997; accepted March 18, 1998.
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This manuscript was sent to Harry A. Fozzard, Consulting Editor, for review by expert referees, editorial decision, and final disposition.
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of JNK by its ability to increase JNK activity when overexpressed\textsuperscript{16} and to inhibit cytokine-induced JNK activity when a dominant negative PAK mutant is transfected into cells.\textsuperscript{17} However, several other kinases that might substitute for PAK function have been implicated in JNK activation. First, members of the mixed-lineage kinase subfamily of serine/threonine kinases have been shown to act downstream of Rac/Cdc42Hs and upstream of mitogen-activated protein kinase/extracellular signal–related kinase (MEKK1)\textsuperscript{18} (a dual leucine zipper-bearing kinase [DLK])\textsuperscript{19} or SEK1/MKK4 (SAPK/JNK).\textsuperscript{20} Second, germinal center kinase (GCK), a member of a second family of Ste20-related kinases, has been shown to activate JNK when overexpressed.\textsuperscript{21} Thus, multiple nonexclusive pathways may stimulate JNK. Because Ang II has recently been shown to stimulate the JAK/STAT pathway as well as JNK, Ang II exhibits similarities in signal events to cytokines such as interleukins, interferons, and TNF. In addition, physiologically, Ang II has cytokine-like properties, such as the ability to stimulate NADH oxidation in VSMCs,\textsuperscript{22} increase TNF production by monocytes,\textsuperscript{23} and regulate cell growth and apoptosis,\textsuperscript{4} that in concert would promote inflammation.

In the present report, we identify αPAK as an upstream activator of JNK in VSMCs stimulated by Ang II. Activation of both PAK and JNK was Ca\textsuperscript{2+} dependent and inhibited by downregulation of phosphol ester–sensitive PKC isoforms. Activation of both PAK and JNK was partially inhibited by tyrosine kinase inhibitors but not by specific Src inhibitors, suggesting that a tyrosine kinase other than c-Src also acts upstream of PAK and JNK in Ang II signaling.

### Materials and Methods

**Materials**

Polyclonal αPAK (sc-881) antibody was purchased from Santa Cruz Biotechnology Inc. GST–c-Jun; glutathione-agarose; and antibodies to PAK-1-NT, PAK-2-NT, and PAK-3-NT were kindly provided by Kinetek Pharmaceuticals Inc. MBP, A23187, thapsigargin, anisomycin, and Ang II were purchased from Sigma Chemical Co. Genistein, tyrphostin 23, and BAPTA-AM were purchased from Calbiochem. Herbinycin A was purchased from Biomol. CP-118,556 (also referred to as PP1) was kindly provided by Pfizer Inc. [γ-\textsuperscript{32}P]ATP was purchased from Amersham. PDBU and GF109203X were purchased from LC Technologies. Mammalian expression vectors containing dominant negative PAK and wild-type tagged JNK were kindly provided by Dr Gary Bokoh (Scripps Research Institute) and Dr Roger Davis (University of Massachusetts), respectively.

**Cell Culture**

VSMCs were isolated from 200- to 250-g male Sprague-Dawley rats and maintained in DMEM supplemented with 10% bovine calf serum, as previously described.\textsuperscript{5} Passage 8 to 15 VSMCs at 80% confluence were grown to confluence and serum starved for 24 hours before use. CHO cells stably transfected with AT1R were kindly obtained from Dr Kenneth Baker (Weis Center for Research) and maintained in F-12 medium supplemented with 20 mmol/L HEPES, 0.2 mg/mL G418, and 10% fetal calf serum. The COS-7 cells were transfected with the pcDNA3.1-AT1, and stably transfected cells were selected by G418. Cells were maintained in DMEM supplemented with 1 mmol/L sodium pyruvate, 0.2 mg/mL G418, and 10% fetal calf serum.

**Immunocomplex MBP In-Gel Kinase Assay**

Growth-arrested VSMCs were stimulated and cells were lysed with lysis buffer containing 10 mmol/L HEPES (pH 7.4), 0.1% Triton X-100, 5 mmol/L EGTA, 5 mmol/L EDTA, 50 mmol/L NaCl, 50 mmol/L NaF, 50 mmol/L sodium pyrophosphate, 1 mmol/L sodium orthovanadate, 10 μg/mL leupeptin, and 1 mmol/L PMSF. Lysates were precleared by centrifugation, and protein concentration was measured by DC protein assay (Bio-Rad). αPAK antibody was added to equal amounts of protein per sample and incubated for 12 hours at 4°C. Antibody complexes were collected by addition of protein A–agarose for 3 hours. Precipitates were washed 5 times in cell lysis buffer, resuspended in SDS sample buffer, and boiled for 10 minutes. After centrifugation for 10 minutes at 10,000g, the supernatants were size fractionated by SDS-PAGE, and αPAK activity was assayed by \textsuperscript{32}P incorporation into MBP using an in-gel kinase assay as previously described.\textsuperscript{3}

**JNK Activity Assay**

Approximately 250 μg of VSMC lysate protein was incubated while constantly rotated with 3 μg GST–c-Jun(1-169) coupled to glutathione–agarose at 4°C for 4 hours. Agarose beads were washed 3 times with buffer B (12.5 mmol/L MOPS [pH 7.2], 12.5 mmol/L β-glycerophosphate, 0.5 mmol/L EGTA, 7.5 mmol/L MgCl\textsubscript{2}, 1 mmol/L DTT, and 0.1% Triton X-100) containing 0.25 mol/L NaCl. Beads were then incubated for 10 minutes at 30°C with buffer B to which 10 mmol/L MgCl\textsubscript{2}, 1 mmol/L MnCl\textsubscript{2}, 50 μmol/L ATP, and 15 μCi of [γ-\textsuperscript{32}P]ATP had been added. In the cotransfection experiments, exogenous FLAG\textsuperscript{TM}-tagged JNK1 was immunoprecipitated with FLAG antibody, and the kinase reaction was performed in buffer B containing 10 mmol/L MgCl\textsubscript{2}, 1 mmol/L MnCl\textsubscript{2}, 50 μmol/L ATP, and 15 μCi of [γ-\textsuperscript{32}P]ATP with 2 μg GST–c-Jun. The reaction was terminated with sample buffer, proteins were separated by SDS-PAGE, and autoradiography was performed.

**Transient Expression of Dominant Negative PAK and Tagged JNK**

COS-AT1R and CHO-AT1R cells were cotransfected with plasmids encoding dominant negative PAK (pCMV6 M-PAK1-K299R)\textsuperscript{13} and FLAG-tagged JNK1 (pcDNA3 M2-JNK1) with the use of LipofectAMINE (Life Technologies). Cells were allowed to recover after transfection for 24 hours in DMEM with 10% serum and were then serum starved for 24 hours before stimulation with Ang II. As a control vector for dominant negative PAK1, cells were transfected with pCMV-lacZ.

**Densiometry and Statistical Analysis**

For quantification of \textsuperscript{32}P incorporation into MBP (PAK) or GST–c-Jun (JNK), autoradiographic films were scanned and analyzed by densitometry with NIH Image 1.59 software. Activation is presented as the fold increase over the respective control (mean ± SEM) based...
on arbitrary densitometry units. All experiments were performed at least 3 times. To test for differences between experimental groups, Student’s t test (unpaired and two tailed) was performed using Statview 512 software. P<0.05 was considered significant.

Results

Activation of JNK and PAK by Ang II in VSMCs

To determine whether Ang II stimulated JNK in VSMCs, cells were treated with 100 nmol/L Ang II, and JNK activity was measured by using GST–c-Jun as the substrate. In accordance with previous reports, Ang II stimulated JNK in VSMCs, with the onset of activity at 10 minutes and a peak at 30 minutes (Figure 1). As a positive control, we used anisomycin, which has previously been shown to be a potent stimulator of JNK (Figure 1).

PAK has been identified as an upstream kinase of JNK that is stimulated by cytokines. To measure PAK activation by Ang II, PAK was immunoprecipitated from cells treated with 100 nmol/L Ang II, and an in-gel-kinase assay with MBP as the substrate was performed. PAK was activated by Ang II within 1 minute, with a peak at 30 minutes (5.2±1.6-fold increase) and sustained activation up to 60 minutes (Figure 2). Similar results were obtained when a PAK immunocomplex MBP in-gel kinase assay was performed as in Figure 2. B, Quantitative densitometry of autoradiograms was performed as described in Materials and Methods.

αPAK Is the Predominant PAK Isoform in VSMCs Stimulated by Ang II

Mammalian tissues contain at least 3 PAK isoforms. αPAK (PAK-1) is highly expressed in the brain, muscle, and spleen. γPAK (PAK-2, hPAK65) is ubiquitously expressed, and βPAK (PAK-3) is highly enriched in the brain. The antibody sc-881 used in this study for immunoprecipitation is directed against αPAK but shows partial cross-reactivity against βPAK and γPAK. Therefore, we determined which PAK isoforms were expressed in VSMCs compared with other cell types known to express specific isoforms. Using isoform-specific PAK antibodies for Western blot analysis, we found that αPAK was the predominant isoform expressed in VSMCs (Figure 4). βPAK was only weakly expressed in VSMCs, whereas γPAK was not expressed in VSMCs but was highly expressed in NIH-3T3 cells (Figure 4). Immunoprecipitation of βPAK and γPAK antibodies and subsequent Western blotting with βPAK and γPAK, respectively, confirmed these results, indicating low-level expression of βPAK only in VSMCs (data not shown). An MBP immunocomplex in vitro kinase assay with βPAK isoform–specific antibodies failed to show βPAK activation by Ang II (data not shown).
Ang II–Mediated Activation of PAK and JNK: Ca\textsuperscript{2+} and PKC Dependence

Ang II has previously been shown to stimulate JNK in a Ca\textsuperscript{2+}-dependent manner in liver epithelial cells\textsuperscript{6} and cardiac myocytes.\textsuperscript{8} Differing results for the PKC dependence of Ang II–mediated JNK activation were reported for these cell systems. Whereas JNK activation by Ang II was PKC independent in liver epithelial cells,\textsuperscript{6} JNK activation was PKC dependent in cardiac myocytes.\textsuperscript{8} The following experiments investigated the dependence of Ang II–mediated JNK and \(\alpha\)PAK activation in VSMCs on Ca\textsuperscript{2+} and PKC. Chelation of extracellular Ca\textsuperscript{2+} only with 1 mmol/L EGTA did not affect \(\alpha\)PAK activation by Ang II. We have recently shown that this protocol completely blocks Ca\textsuperscript{2+} entry into VSMCs but has no effect on the rapid increase in Ca\textsuperscript{2+} stimulated by Ang II.\textsuperscript{24} However, chelation of intracellular Ca\textsuperscript{2+} with BAPTA-AM in the presence of 1 mmol/L EGTA (which completely blocks Ang II–mediated changes in intracellular Ca\textsuperscript{2+}) [Reference 24]) abolished \(\alpha\)PAK activation (Figure 5A, upper panel). Increasing intracellular Ca\textsuperscript{2+} by treating VSMCs with 10 mmol/L A23187 for 15 minutes stimulated \(\alpha\)PAK \(\approx 2\)-fold (data not shown) compared with the \(\approx 4\)-fold activation observed for Ang II. Similar results were obtained for activation of JNK, in that Ca\textsuperscript{2+} chelation with EGTA did not inhibit Ang II–stimulated JNK activity, whereas Ca\textsuperscript{2+} chelation with BAPTA-AM in the presence of 1 mmol/L EGTA inhibited JNK activity (Figure 5A, lower panel). It should be noted that the increase in JNK activity by Ang II after preincubation in Ca\textsuperscript{2+}-free HBSS buffer (DMEM control in Figure 5) was smaller than after preincubation in DMEM supplemented with 0.4% fetal calf serum (eg, Figure 1). Furthermore, BAPTA-AM pretreatment alone increased activity of JNK in VSMCs (Figure 5A, lower panel). This phenomenon has previously been reported for the activation of JNK\textsuperscript{2} and has been interpreted as a stress-related or toxic effect of BAPTA-AM.\textsuperscript{6}

We next determined the effect of downregulation of phorbol ester–binding PKC isoforms on \(\alpha\)PAK and JNK activation by Ang II and PMA. PMA alone (1 mmol/L) activated \(\alpha\)PAK and JNK \(\approx 2\)-fold (Figure 6A and 6B) compared with the 4-fold and 2.5-fold increases, respectively, with Ang II, indicating that signals in addition to PKC are required for full activation of \(\alpha\)PAK and JNK. Downregulation of PKC by pretreatment with 1 mmol/L PDBU, which inhibits the phorbol ester–binding PKC isoforms (PKC-\(\alpha\), -\(\beta\), -\(\gamma\), -\(\delta\), -\(\epsilon\), -\(\theta\), and -\(\eta\)) for 24 hours inhibited Ang II– and PMA-mediated activation of \(\alpha\)PAK and JNK (Figure 6A). Pretreatment with 1 \(\mu\)mol/L GF109203X for 10 minutes (an inhibitor of PKC-\(\alpha\), -\(\beta\), -\(\gamma\), -\(\delta\), and -\(\epsilon\)) did not affect \(\alpha\)PAK and JNK activation by Ang II (Figure 6A), whereas PMA-mediated \(\alpha\)PAK and JNK activation was diminished. Interestingly, Eguchi et al\textsuperscript{26} reported that Ang II–mediated activation of ERK1/2 in VSMCs could be inhibited by PMA pretreatment, whereas GF109203X had no effect on ERK1/2 activation. Because PDBU downregulation inhibited JNK activation but GF109203X had no effect, these findings suggest that the PKC isoforms involved in \(\alpha\)PAK and JNK activation by Ang II in VSMC are most likely PKC-\(\theta\) or -\(\eta\).

Activation of \(\alpha\)PAK and JNK by Ang II Is Dependent on a Tyrosine Kinase Other Than Src

Tyrosine kinases have been implicated as upstream mediators of Ang II–induced JNK activation by the following findings. First, in GN4 liver epithelial cells JNK activation by Ang II could be inhibited by the tyrosine kinase inhibitor genistein.\textsuperscript{6} Second, after comparing different liver epithelial cell lines, this same group of investigators reported a close correlation between activation of JNK and activation of the recently identified CADTK, which is the rat homolog of the human PYK2 tyrosine kinase. More recently, Brinson et al\textsuperscript{27} showed that Ang II stimulated CADTK in VSMCs in a calcium- and PKC-dependent manner. We have previously demonstrated that Ang II activates the cellular tyrosine kinase c-Src and

**Figure 4.** \(\alpha\)PAK is the predominant PAK isof orm in VSMCs. Western blotting with isoform-specific PAK antibodies was performed with VSMCs, NIH-3T3 cells, and rat brain. Respective isoforms are indicated by arrowheads. VSMCs primarily express \(\alpha\)PAK.
have shown that c-Src activation is critical for ERK1/2 stimulation by Ang II. Moreover, v-Src has been implicated in activation of JNK in transiently transfected NIH-3T3 cells. Therefore, we determined the effect of different tyrosine kinase inhibitors on Ang II–mediated activation of ERK1/2 and JNK activation by Ang II. Pretreatment of VSMCs with 100 μmol/L genistein for 1 hour (Figure 7) or with 100 μmol/L tyrphostin G 23 for 16 hours (data not shown) partially inhibited αPAK and JNK activation by Ang II. To address the role of c-Src more specifically, we investigated the effect of the tyrosine kinase inhibitor PP1, which has been reported to inhibit Src-family tyrosine kinases preferentially. We have recently characterized the inhibitory effects of PP1 in vitro and found that c-Src, immunoprecipitated from VSMCs after Ang II stimulation, was inhibited by PP1, with an IC50 of 1.32±0.27 μmol/L. Furthermore, we demonstrated that 10 μmol/L PP1 inhibited ERK1/2 activation by Ang II. To examine the effect of Src inhibition by PP1 on αPAK and JNK activation by Ang II, we pretreated VSMCs for 15 minutes and then measured αPAK and JNK activity. Neither αPAK nor JNK activation by Ang II was inhibited by 50 μmol/L PP1 (Figure 7). Furthermore, pretreatment of VSMCs with 1 μmol/L herbimycin A, which is also thought to inhibit c-Src preferentially, had no effect on αPAK and JNK activation by Ang II (data not shown). These data indicate that c-Src is not the tyrosine kinase required for Ang II activation of JNK.

Dominant Negative PAK Inhibits the Activation of JNK by Ang II

To examine further whether αPAK is upstream of JNK in Ang II–mediated signaling, we introduced dominant negative PAK and FLAG-tagged-JNK into CHO-AT1R and COS-AT1R cells and measured activation of JNK. In both CHO-AT1R and COS-AT1R cells cotransfected with lacZ and FLAG-tagged-JNK, exogenous (FLAG tagged) JNK was activated by 100 nmol/L Ang II (Figure 8). In the CHO-AT1R cells cotransfected with dominant negative PAK and FLAG-tagged-JNK, JNK activation by Ang II was completely inhibited. Similarly, in the COS-AT1R cells cotransfected with dominant negative PAK and FLAG-tagged JNK, JNK activation by Ang II was significantly inhibited. These results demonstrate that PAK is upstream of JNK in an Ang II–mediated signaling pathway.

Discussion

The major finding of this study is that Ang II–mediated activation of αPAK is required for stimulation of JNK in VSMCs. Our data further indicate that activation of PKC and a tyrosine kinase, together with mobilization of intracellular calcium, are required for Ang II–mediated αPAK and JNK activation. The following results suggest that αPAK acts upstream of JNK in Ang II signaling. Activation of αPAK preceded activation of JNK by Ang II in VSMCs. Activation of both αPAK and JNK appeared to be partially dependent on agonist-induced release of intracellular Ca2+. Downregulation of phorbol ester–sensitive PKC isoforms by PDBU or inhib.
bition of PKC-α, -β, -γ, -δ, and -ε by GF109203X had similar effects on αPAK and JNK activation, in that PDBU pretreatment inhibited αPAK and JNK activation by Ang II, whereas pretreatment with GF109203X did not. Different tyrosine kinase inhibitors had the same inhibitory effects on αPAK and JNK activation, with inhibition by genistein and tyrphostin 23 and lack of inhibition by Src-selective tyrosine kinase inhibitors herbimycin A and PPI. Most importantly, in CHO and COS cells stably transfected with the AT1R, activation of JNK by Ang II was inhibited by transfection with dominant negative PKA. Although CHO and COS cells exhibited differences in signal transduction compared with VSMCs in several respects, they are a useful model system. In fact, we found that Ang II stimulated JNK (this study) and ERK1/2 (M. Ishida, unpublished observations, 1997) to the same magnitude and with a similar time course as VSMCs.

Activation of JNK has been reported to be positively and negatively regulated by PKC, depending on the cell type and stimulus investigated. Kudoh et al demonstrated in cardiac myocytes that Ang II–stimulated JNK activation was inhibited by downregulation of PKC with PMA. In contrast, Zohn et al reported that the Ang II–mediated JNK activation in GN4 liver epithelial cells was potentiated by downregulation of PKC by PMA pretreatment. Similarly, Cadwallader et al reported that endothelin-1–mediated JNK activation in Rat-1 fibroblasts was negatively regulated by PKC. The Ca2+-dependent and phorbol ester–sensitive αPAK and JNK activation by Ang II observed in the present study suggests that a classic PKC isoform is involved in VSMCs. However, when we tested GF109203X, a PKC inhibitor that is thought to inhibit PKC-α, -β, -γ, -δ, and -ε preferentially, we saw no inhibitory effect on αPAK or JNK activation by Ang II, whereas PMA-induced αPAK and JNK stimulation was inhibited (Figure 6). These data suggest that novel PKC isoforms (PKC-θ and -η) are upstream regulators of αPAK and JNK in Ang II signaling. Because these isoforms are Ca2+-insensitive, the data suggest that the Ca2+-dependence observed for αPAK and JNK is due to another mediator (eg, a calcium-dependent tyrosine kinase).

Tyrosine kinases implicated in JNK activation include PYK2 (also termed CADTK) and Src. A role for CADTK is implied by the findings that overexpression of PYK2 stimulated JNK and that dominant negative mutants of Rac/Cdc42Hs effectively blocked PYK2-mediated JNK activation. Moreover, Yu et al demonstrated that activation of JNK by Ang II was correlated with the activation of CADTK in liver epithelial cells. Brinson et al recently demonstrated that CADTK was expressed in VSMCs and stimulated by Ang II in a Ca2+- and PKC-dependent manner. Finally, several other reports have shown that CADTK activation is PKC dependent. A role for Src is implied by the findings that transient transfection of NIH-3T3 cells with v-Src increased JNK activity. Also, v-Src–mediated JNK activation was inhibited by coexpression of dominant negative Rac/Cdc42Hs and dominant negative PAK, placing Src upstream of Rac/Cdc42Hs and hence, of PAK. Recent data from our laboratory have shown that c-Src activation is critical for Ang II–induced ERK1/2 activation. However, we found no evidence to support a role for c-Src in JNK activation by Ang II in VSMCs on the basis of studies with pharmacological inhibitors. While it is tempting to speculate that CADTK, PAK, and JNK constitute components of the same Ang II–dependent signaling pathway, future studies will be required to demonstrate this pathway in VSMCs.

The present data combined with recent findings from our laboratory regarding ERK1/2 activation support the following model for Ang II–mediated ERK1/2 and JNK activation in VSMCs (Figure 9). ERK1/2 activation occurs via a Ca2+-independent pathway that involves c-Src and the atypical PKC isoform PKC-ζ. In contrast, JNK activation occurs via a Ca2+-dependent pathway that involves a tyrosine kinase other than Src and a novel PKC isoform. We do not know the temporal sequence of activation of these upstream mediators for JNK. Important areas for future investigations include identification of the tyrosine kinase and PKC isoform involved in JNK activation. In addition, defining the roles of JNK and ERK1/2 in Ang II–mediated effects on VSMC function will be important. The present study, in combination with previous reports that Ang II stimulates the JAK/STAT pathway, supports the emerging role of Ang II as a proinflammatory mediator for cardiovascular tissues. It is clear that understanding the in vivo consequences of Ang II cytokine-like actions may provide insights into the pathogenesis of cardiovascular disease.

Acknowledgments
This study was supported by grants from the Deutsche Forschungsgemeinschaft to Dr Schmitz (SCHR 117/4-2), the National Institutes of Health (Bethesda, Md) to Dr Berk (RO1 HL 44721), and the Heart and Stroke Foundation of British Columbia and Yukon to Dr Pelech. Dr Berk was an Established Investigator of the American Heart Association during performance of this research. Dr Pelech was the recipient of a Medical Research Council of Canada Industrial Scientist Award.
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Circ Res. 1998;82:1272-1278
doi: 10.1161/01.RES.82.12.1272

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