p38 MAP kinase pathway regulates angiotensin II-induced contraction of rat vascular smooth muscle

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Meloche, Sylvain, Jacques Landry, Jacques Huot, François Houle, François Marceau, and Edith Giasson. p38 MAP kinase pathway regulates angiotensin II-induced contraction of rat vascular smooth muscle. Am J Physiol Heart Circ Physiol 279: H741–H751, 2000.—Angiotensin II (ANG II) is a multifunctional hormone that exerts potent vasoconstrictor and hypertrophic effects on vascular smooth muscle. Here, we demonstrate that the p38 mitogen-activated protein (MAP) kinase pathway is involved in ANG II-induced vascular contraction. Addition of ANG II to rat aortic smooth muscle cells (SMC) caused a rapid and transient increase of p38 activity through activation of the AT1 receptor subtype. This response to ANG II was strongly attenuated by pretreating cells with antioxidants and diphenylene iodonium and was mimicked by exposure of cells to H2O2. Stimulation of p38 by ANG II resulted in the enzymatic activation of MAP kinase-activated protein (MAPKAP) kinase-2 and the phosphorylation of heat shock protein 27 (HSP27) in aortic SMC. Pretreatment of cells with the specific p38 MAP kinase inhibitor SB-203580 completely blocked the ANG II-dependent activation of MAPKAP kinase-2 and phosphorylation of HSP27. ANG II also caused a robust activation of MAPKAP kinase-2 in the intact rat aorta. Incubation with SB-203580 significantly decreased the potency of ANG II to induce contraction of rat aortic rings and depressed the maximal hormone response. These results suggest that the p38 MAP kinase pathway selectively modulates the vasoconstrictor action of ANG II in vascular smooth muscle.

smooth muscle cell; signal transduction; mitogen-activated protein kinase; angiotensin receptor

ANGIOTENSIN II (ANG II), the primary active component of the renin-angiotensin system, plays an important role in vascular function. It was originally identified as a potent vasoconstrictor hormone whose response involved both a direct action on vascular smooth muscle and an indirect effect mediated by the sympathetic nervous system (10). More recently, ANG II has been shown to stimulate protein synthesis and induce cellular hypertrophy in vascular smooth muscle cells (SMC) (8, 22, 23) and to promote vascular cell migration (18). The cellular mechanisms underlying these diverse actions of ANG II are not clearly understood but are likely to involve the activation of distinct signaling pathways. All of the vascular effects of ANG II are mediated by the G protein-coupled AT1 receptor subtype. Binding of ANG II to the AT1 receptor activates multiple heterotrimeric G proteins that link the receptor to the stimulation of phospholipases C and D and to the inhibition of adenyl cyclase (11, 25). These early biochemical events ultimately lead to the activation of complex cascades of protein serine/threonine kinases.

Among the serine/threonine kinases that are commonly employed to transduce extracellular signals are the mitogen-activated protein (MAP) kinases. Three subfamilies of MAP kinases have been extensively characterized in mammalian cells: the extracellular signal-regulated kinases (ERKs), the c-Jun NH2-terminal kinases (JNKs)/stress-activated protein kinases (SAPKs), and p38 (16, 56, 57). All these MAP kinases are activated by dual phosphorylation of tyrosine and threonine residues within the regulatory motif Thr-X-Tyr, catalyzed by a specific MAP kinase family member. The ERK isoforms ERK1 and ERK2 are activated predominantly by growth factors, differentiation stimuli, phorbol esters, and Ca2+ (34). Once activated, ERK1 and ERK2 can phosphorylate numerous cytoplasmic and nuclear proteins, including the protein kinases p90RSK and Mnk1/Mnk2, stathmin, and the transcription factor Elk-1 (16, 56). Activation of the ERK pathway is associated with the mitogenic response to growth factors and the differentiation of specific cell lineages (49). In contrast, the JNK and p38 MAP kinase pathways are strongly activated by cellular stresses (heat and osmotic shock, ultraviolet irradiation, inhibition of protein synthesis), endotoxic lipopolysaccharide, inflammatory cytokines, and chemotactic peptides (33). JNK exists in multiple isoforms (8, 22, 23) and to promote vascular cell migration (18).
have been shown to phosphorylate and activate the transcription factors c-Jun, Elk-1, and ATF2 (16, 56). The other stress-activated MAP kinase pathway is comprised of p38 (also known as RK, P40, CSBP, and Mxi2) and the related homologs p38β (also termed p38-2), p38γ (also termed ERK6 and SAPK3), and p38δ (also termed SAPK4) (see Ref. 57 and references therein). Physiological substrates of p38 and p38β include the protein kinases MAP kinase-activated protein (MAPKAP) kinase-2, MAPKAP kinase-3, and Mnk1/Mnk2 as well as the transcription factors CHOP/GADD153 and Elk-1 (16, 56). The related kinases MAPKAP kinase-2 and MAPKAP kinase-3 phosphorylate the small heat shock protein HSP27 in vivo (20, 28, 40, 50). The physiological substrates of p38γ and p38δ remain to be identified. Activation of the JNK and p38 MAP kinase pathways is mainly associated with the response to stress and inflammation (33).

Here, we report that the G protein-coupled receptor agonist ANG II activates p38 MAP kinase in cultured aortic SMC and in the intact aorta, leading to activation of MAPKAP kinase-2 and phosphorylation of HSP27. The enzymatic activation of p38 is mediated by the AT1 receptor and is dependent on the generation of HSP27. The enzymatic activation of p38 is mediated by the reaction of MAPKAP kinase-2 and phosphorylation of aortic SMC and in the intact aorta, leading to activation of MAPKAP kinase-2 and phosphorylation of HSP27 in vivo (20, 28, 40, 50). The physiological substrates of p38γ and p38δ remain to be identified. Activation of the JNK and p38 MAP kinase pathways is mainly associated with the response to stress and inflammation (33).

HSP27 phosphorylation. Quiescent aortic SMC in 30-mm dishes were metabolically labeled for 4 h at 37°C in phosphate-free medium containing 25 μCi/ml [32P]phosphoric acid. The cells were stimulated by addition of ANG II to the medium for 30 min. Cell lysates were prepared and subjected to immunoprecipitation with anti-MAPKAP kinase-2 antibody. The precipitates were collected by incubation with 15 μl of protein A-Sepharose (50% vol/vol in buffer I) for 30 min. The immune complexes were washed three times with buffer I and resuspended in 25 μl of kinase buffer K (20 mM MOPS, pH 7.0, 10% glycerol, 15 mM MgCl2, 1 mM DTT, 40 mM p-nitrophenyl phosphate, 1 mM leupeptin, 0.1 mM PMSF, 0.05% Triton X-100, and 0.3 μg of protein kinase A inhibitor) containing 2.5 μg of recombinant HSP27, 100 μM ATP, and 3 μCi [γ-32P]ATP. The reactions were incubated at 30°C for 30 min and terminated by addition of sample buffer. The phosphoprotein fraction of the substrate protein was examined after SDS gel electrophoresis by autoradiography and quantified by densitometry using the National Institutes of Health (NIH) Image software.

Protein synthesis measurements. Quiescent aortic SMC in 24-well plates were stimulated with 100 nM ANG II in serum-free quiescence medium containing 0.5 μCi/ml [3H]leucine. After 24 h of stimulation, the medium was aspirated and the cells were incubated for a minimum of 30 min in cold 5% trichloroacetic acid. The cells were washed once with trichloroacetic acid and three times with tap water. The radioactivity incorporated into trichloroacetic acid-precipitable material was measured by liquid scintillation counting after solubilization in 0.1 M NaOH. For experiments with SB-203580, quiescent cells were pretreated for...
30 min with the indicated concentrations of SB-203580 and stimulated for 24 h with ANG II in the continuous presence of the inhibitor.

**Contractility studies.** The thoracic aorta was removed from male Fisher 344 rats (300–350 g) killed by breathing 100% CO2; placed in oxygenated Krebs solution at room temperature; cleared of adherent fat, blood, and excess connective tissue; and transversally cut to produce rings ~3 mm wide. The endothelium was removed by gentle, repeated rubbing of the vessel lumen with a metal rod. Each ring was suspended between a metal hook and a thread loop under a tension of 1 g in a 5-ml tissue bath containing oxygenated (95% O2–5% CO2) and warmed (37°C) Krebs solution. The composition of Krebs was (in mM) 117.5 NaCl, 4.7 KCl, 1.2 KH2PO4, 1.18 MgSO4, 2.5 CaCl2, 25.0 NaHCO3, and 5.5 D-glucose. Isometric changes of the vascular tone were measured using a force transducer (model 52–9545, Harvard) coupled to an LKB chart recorder (model 2210 or REC 102).

Contractility studies were based on the construction of full cumulative concentration-response curves for the agonists phenylephrine, added 1 h after the beginning of tissue incubation, and ANG II, tested at time 3 h. The tissues were washed extensively with fresh Krebs after the maximal effect was reached. For experiments with SB-203580 and DPI, the inhibitor or vehicle was added to the bathing solution 30 min before agonist treatment. Contractility results are expressed as the mean absolute force of contraction in grams. The concentration-response curves are characterized by the half-maximal effective concentration and the maximal absolute force of contraction. Statistical analysis was by Mann-Whitney test using InStat 2.0 software (GraphPad Software).

**Other methods.** Dose-response curves were analyzed according to a four-parameter logistic equation using the ALLFIT computer program (17).

**RESULTS**

**ANG II stimulates p38 MAP kinase activity in aortic SMC.** To explore the role of p38 MAP kinase in the physiological actions of ANG II on blood vessels, we first tested the ability of the hormone to regulate the activity of p38 in cultured cells. Rat aortic SMC were made quiescent by serum deprivation and then treated with ANG II, and p38 was immunoprecipitated from cell lysates. The phosphotransferase activity of the enzyme was assayed directly using GST-ATF2 as substrate. Addition of ANG II caused a rapid activation of p38, which reached a maximum at 5 min and returned to near basal level after 120 min (Fig. 1A). This time course of p38 activation contrasts with that observed in response to cellular stresses, in which activation of the enzyme is slower and more sustained (J. C. Scimeca and S. Meloche, unpublished observations; Ref. 46). To determine the subtype of ANG II receptors involved in the activation of p38, the cells were pretreated with selective receptor antagonists before ANG II stimulation. Figure 1B shows that incubation with the AT1-selective antagonist losartan completely abolished p38 activation, whereas the AT2 antagonist PD-123319 had no effect. These results demonstrate that ANG II activates p38 MAP kinase through the G protein-coupled AT1 receptor in vascular SMC.

**Lack of involvement of classic AT1 receptor signaling pathways in the activation of p38 MAP kinase by ANG II.** In contrast to the ERK pathway, very little is known about the early signaling events that trigger activation of the p38 MAP kinase pathway. We used a pharmacological approach to characterize the signaling path-
ways coupling the AT₁ receptor to activation of p38 in vascular SMC. The best documented signaling pathway of the AT₁ receptor is the stimulation of phospholipase C, which generates the second messengers diacylglycerol and inositol 1,4,5-trisphosphate (11, 25). Diacylglycerol is a physiological activator of conventional and novel PKC isoforms, whereas inositol 1,4,5-trisphosphate binds to specific receptor channels to release Ca²⁺ from intracellular stores. We therefore investigated the role of PKC and Ca²⁺ in the stimulatory effect of ANG II on p38 enzymatic activity. As shown in Fig. 2A, treatment of cells with CGP-41251 and GF-109203X, compounds that inhibit conventional and novel PKC isoforms, or with Go-6976, a more selective inhibitor of conventional Ca²⁺-dependent PKC isoforms, had no effect on ANG II-dependent activation of p38. The role of Ca²⁺ was analyzed by incubating the cells with the membrane-permeable Ca²⁺ chelator BAPTA-AM. Chelation of intracellular Ca²⁺ did not inhibit the activation of p38 by ANG II but, in contrast, potentiated the basal and hormone-stimulated activity of the enzyme. To better define the role of Ca²⁺ in the regulation of p38, we examined the effect of BAPTA-AM on the time course of activation of p38. Interestingly, treatment with BAPTA-AM not only enhanced the activity of p38 in resting cells but also prevented the rapid inactivation of the kinase in ANG II-stimulated cells (Fig. 3). These results suggest that p38 is negatively regulated by a Ca²⁺-dependent protein phosphatase that is constitutively present in aortic SMC. In this regard, we have recently shown that Ca²⁺ chelation completely abolishes the induction of MAP kinase phosphatase-1 expression in aortic SMC (52), thereby suggesting that a member of the dual-specificity phosphatase family may be responsible for the inactivation of p38 in these cells.

In common with other G protein-coupled receptor agonists, ANG II stimulates tyrosine phosphorylation of multiple substrates in target cells, including vascular SMC (38). Recent observations made in various cellular systems have implicated protein tyrosine kinases in the activation of the ERK MAP kinase pathway by G protein-coupled receptors (55). We therefore asked whether activation of a cellular tyrosine kinase was required for ANG II-dependent activation of p38. Figure 2B shows that treatment of aortic SMC with the chemically and mechanically distinct broad spectrum tyrosine kinase inhibitors genistein and herbimycin A did not prevent the activation of p38 by the hormone. Similarly, treatment of cells with the Src family-selective tyrosine kinase inhibitor PP1 or the Jak2 inhibitor AG490 did not interfere with p38 activation (Fig. 2C).
In a recent study, it was reported that tyrosine phosphorylation of p38 in response to the G protein-coupled receptor agonist N-formyl-Met-Leu-Phe is partially blocked by the phosphatidylinositol 3-kinase inhibitor wortmannin in neutrophils (32). Because it was recently shown that ANG II stimulates phosphatidylinositol 3-kinase activity in vascular SMC (E. Giasson and S. Meloche, unpublished observations; Ref. 51), we tested the possibility that p38 is a downstream target of this signaling pathway. However, treatment of cells with wortmannin or LY-294002, two structurally different inhibitors of phosphatidylinositol 3-kinase, did not inhibit ANG II-dependent activation of p38 (Fig. 2D). Taken together, these data indicate that ANG II stimulates p38 enzymatic activity by a pathway distinct from the classic AT1 receptor signaling pathways.

Generation of ROS is necessary for ANG II-dependent activation of p38 in aortic SMC. Accumulating evidence indicates that ROS may function as intracellular second messengers in receptor signaling pathways (19, 35). Specifically, treatment of various cell types with oxidizing agents has been shown to activate the MAP kinase family members ERK1/ERK2 (5, 26), JNK (14, 26), and p38 (14, 26, 27). In light of these observations, we next sought to determine whether the activation of p38 by ANG II might be dependent on the production of ROS. To address this question, we first incubated aortic SMC with the antioxidants NAC and GSH (3, 41) before ANG II stimulation. Figure 4A shows that both NAC and GSH strongly attenuated ANG II-dependent activation of p38. Previous studies have demonstrated the existence of a membrane-associated NADH/NADPH oxidase system in vascular SMC (24, 43). The activity of this oxidase increases in response to ANG II stimulation (24). To determine whether the vascular NADH/NADPH oxidase might be involved in the activation of p38 by ANG II, we tested the effect of DPI, a potent inhibitor of flavin-containing enzymes (44). Incubation of aortic SMC with DPI markedly inhibited ANG II-stimulated p38 activity, whereas the mitochon-
drial NADH dehydrogenase inhibitor rotenone had no effect (Fig. 4B). To directly test the hypothesis that ROS can activate p38, the cells were exposed to the oxidant H$_2$O$_2$. Addition of H$_2$O$_2$ potently stimulated the enzymatic activity of p38, resulting in sixfold activation at 15 min (Fig. 4C). These results are consistent with recent findings by Ushio-Fukai et al. (53) and strongly suggest that ROS act as second messengers for ANG II in mediating the activation of p38 in vascular SMC.

**ANG II stimulates MAPKAP kinase-2 activity and HSP27 phosphorylation in aortic SMC.** We next wanted to determine whether the stimulation of p38 by ANG II results in the activation of MAPKAP kinase-2, a known physiological substrate of the enzyme (20, 50). Aortic SMC were stimulated with ANG II, and the activity of MAPKAP kinase-2 was determined by immune complex kinase assay using recombinant HSP27 as substrate. Addition of ANG II caused a 17-fold increase in MAPKAP kinase-2 activity at 5 min (Fig. 5A). This effect of ANG II was completely abolished by preincubating the cells with the pyridinyl imidazole compound SB-203580, a highly specific inhibitor of p38 and p38β enzymes (29, 39). The effect of SB-203580 was dose dependent, with an IC$_{50}$ value of 0.10 ± 0.03 μM and a maximal inhibitory effect observed at ~10 μM (Fig. 5A). In contrast, incubation of cells with PD-98059, a selective inhibitor of the ERK pathway, did not interfere with MAPKAP kinase-2 activation (data not shown).

Activation of MAPKAP kinase-2 leads to phosphorylation of HSP27 in diverse cell types (20, 28, 50). To examine the effect of ANG II on the phosphorylation of HSP27, aortic SMC were metabolically labeled with $^{32}$P$_i$ and stimulated with ANG II, and HSP27 was immunoprecipitated from cell lysates before analysis by SDS gel electrophoresis. Treatment with ANG II significantly increased the phosphorylation of HSP27, and this effect was completely suppressed by preincubating the cells with SB-203580 (Fig. 5B). These results demonstrate that ANG II activates a p38 MAP kinase cascade that leads to phosphorylation of HSP27 in vascular SMC.

**Involvement of the p38 MAP kinase pathway in ANG II-induced vascular contraction.** To evaluate the biological significance of p38 activation by ANG II, we tested the effect of SB-203580 on the hypertrophic and vasoconstrictor action of the hormone. The hypertrophic effect of ANG II was evaluated by measuring the rate of protein synthesis in cultured rat aortic SMC. For these experiments, quiescent cells were preincubated with increasing concentrations of SB-203580 before stimulation with ANG II for 24 h in the continuous presence of the inhibitor. Figure 6 shows that SB-203580 had little effect on ANG II-induced protein synthesis up to a concentration of 10 μM, where it partially inhibited the response of the hormone. The IC$_{50}$ value of SB-203580 for inhibition of protein synthesis was ~100-fold higher than that observed for inhibition of MAPKAP kinase-2 (Fig. 5A). This finding is not consistent with a major involvement of p38 in the regulation of protein synthesis by ANG II.

The role of the p38 MAP kinase pathway in vascular smooth muscle contraction was investigated using fresh rat aortic rings. To extrapolate the results obtained in cultured aortic SMC to the intact aorta, we first examined the ability of ANG II to activate the p38 pathway in intact aortic rings. To this end, aortic rings were stimulated with ANG II for different times and then frozen in liquid N$_2$ before assay for MAPKAP kinase-2 activity. As shown in Fig. 7, ANG II caused a robust activation of MAPKAP kinase-2 in the intact aorta, with kinetics comparable to those seen in cultured aortic SMC. Preincubation of aortas with 10 μM SB-203580 nearly abolished ANG II-dependent activation of the enzyme. As expected, ANG II induced a strong contraction of the rat aorta, with a half-maximal
effect observed at 6.6 ± 0.9 nM (Fig. 8B). Incubation with SB-203580 significantly shifted the concentration-response curve of ANG II to the right (IC_{50} = 15.5 ± 4.7 nM, P < 0.02) and reduced maximal ANG II-induced contraction. The effect of SB-203580 was dose dependent, with 50% inhibition of ANG II-induced contraction observed at 0.17 ± 0.09 μM (Fig. 8B). By contrast, SB-203580 had no effect on the apparent potency or maximal response of the α-adrenergic agonist phenylephrine (Fig. 8A). These results provide strong evidence that a p38 MAP kinase modulates the vasoconstrictor action of ANG II in vascular smooth muscle.

Because the stimulation of p38 activity by ANG II is dependent on the generation of ROS (Fig. 4) (53), we also tested the effect of DPI on ANG II-induced contraction of the rat aorta. Preincubation with 20 μM DPI significantly depressed maximal ANG II-induced contraction (P < 0.03) and shifted the concentration-response curve of the hormone rightward (Fig. 9).

**DISCUSSION**

ANG II is a pleiotropic hormone that exerts a wide spectrum of actions on the cardiovascular system through activation of multiple signaling pathways. In this paper, we report that ANG II activates the p38 MAP kinase pathway in rat aortic smooth muscle and that this pathway is involved in the vasoconstrictor action of the hormone. ANG II, working through the AT_1 receptor subtype, stimulated the enzymatic activ-
and tumor necrosis factor–lipopolysaccharide-induced synthesis of interleukin-1, which was initially developed as an inhibitor of p38 and p38β derivative that inhibits the kinase activity of p38 (39). The development of small cell-permeant drug inhibitors has been crucial for the identification of the biological roles and physiological substrates of p38 MAP kinase isoforms. SB-203580 is a pyridinyl imidazole partial inhibitor. Each value represents the mean ± SE of 4 determinations. See text for statistical analysis.

Fig. 9. Effect of DPI on ANG II-induced contraction of rat aorta. Rat aortic rings were preincubated for 30 min with vehicle alone or with 20 μM DPI before addition of ANG II. Full cumulative concentration-response curves were constructed in the continuous presence of the inhibitor. Each value represents the mean ± SE of 4 determinations. In vivo studies demonstrated that SB-203580 potently inhibits inflammatory cytokines productions but also in response to stimulation with a variety of ROS in vascular SMC (24) and in intact rat aortic ring segments (47). Also, during the course of this study, Ushio-Fukai et al. (53) reported that ANG IIstimulates phosphorylation of p38 in vascular SMC and that treatment with DPI or expression of catalase partially inhibits ANG II-dependent phosphorylation of the kinase. Third, addition of the exogenous oxidant H2O2 mimics the activation of p38 by ANG II. As for many other signaling pathways, the direct cellular targets of ROS in the p38 MAP kinase pathway remain to be identified.

There is now considerable evidence that low concentrations of ROS can function as classic second messenger molecules (19, 35). ROS are produced by every cell type not only as by-products of electron transfer reactions but also in response to stimulation with a variety of cytokines and growth factors such as tumor necrosis factor-α, interleukin-1, platelet-derived growth factor, epidermal growth factor, and ANG II (19, 35). The mechanism responsible for the agonist-dependent generation of ROS in nonphagocytic cells remains to be clarified. However, recent work suggests that a membrane-associated NADH/NADPH oxidase system similar to the neutrophil NADPH oxidase may be the primary source of ROS in vascular tissue (24, 43, 45). The presence of p22phox, a subunit of cytochrome b558, has been demonstrated in vascular SMC (21). As mentioned above, ANG II increases production of ROS via an NADH/NADPH-dependent membrane-bound oxidase system in cultured aortic SMC and in the intact aorta (24, 47). Importantly, treatment with DPI (24), a

203580 inhibits the activity of both p38 and p38β, we cannot draw a firm conclusion on the relative contribution of these p38 MAP kinase isoforms to vascular smooth muscle contraction at this stage. However, the fact that ANG II stimulates the activity of p38 in cultured vascular SMC supports the hypothesis that p38 is involved in that response. Importantly, these findings suggest that SB-203580 and related compounds may show therapeutic usefulness in conditions other than chronic inflammatory diseases.

The regulatory pathways that lead to activation of p38 MAP kinase are still poorly defined in mammalian cells. p38 is phosphorylated and activated by the dual-specificity kinases MKK3 and MKK6 (57). In this study, we used a pharmacological approach to identify the early signaling events that are responsible for coupling the AT1 receptor to the activation of p38. None of the conventional intracellular signals commonly activated by Gα- or Gβγ-coupled receptors were found to be involved in the stimulation of p38 activity. However, the following observations provide strong evidence for a role of ROS as second messengers in the p38 activation pathway. First, ANG II-dependent p38 activation is inhibited by pretreatment of cells with the antioxidants NAC and GSH. NAC can directly react with free radicals but also increases the intracellular concentration of GSH (3). Second, the activation of p38 by ANG II is markedly attenuated by the NADH/NADPH oxidase inhibitor DPI. Previous studies have shown that DPI inhibits ANG II-stimulated production of ROS in vascular SMC (24) and in intact rat aortic rings (47). Also, during the course of this study, Ushio-Fukai et al. (53) reported that ANG II stimulates phosphorylation of p38 in vascular SMC and that treatment with DPI or expression of catalase partially inhibits ANG II-dependent phosphorylation of the kinase. Third, addition of the exogenous oxidant H2O2 mimics the activation of p38 by ANG II. As for many other signaling pathways, the direct cellular targets of ROS in the p38 MAP kinase pathway remain to be identified.

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pharmacological inhibitor of the flavoprotein component of NADH/NADPH oxidase, or inhibition of p22phox expression by an antisense approach (54) significantly decreases ANG II-stimulated ROS production in vascular SMC. The increase of ROS levels has been associated with the induction/activation of transcription factors (13), the activation of MAP kinase isoforms (5, 14, 26, 27), and the inhibition of tyrosine phosphatases (30). Interestingly, it has been reported that ROS exert contractile effects on SMC of vascular origin (4, 48). In the present study, we found that DPI significantly reduces ANG II-induced contraction of the aorta. The hypertensive response induced by ANG II infusion in the rat was also found to be associated with increased vascular superoxide production, thereby highlighting the physiopathological importance of NADH/NADPH oxidase-derived ROS in the regulation of vascular tone and arterial pressure (47). Together, these observations are consistent with the idea that activation of the p38 pathway may link the ANG II-dependent production of ROS to vascular contraction.

The mechanism by which activation of the p38 MAP kinase pathway modulates the contractile response to ANG II remains to be established. One possibility is that HSP27 directly regulates the cycling of myosin cross bridges along actin filaments through its ability to interact with actin. HSP27 behaves as an actin cap-binding protein in vitro and is able to inhibit actin polymerization in vinculin-rich fraction of turkey gizzard smooth muscle (42). In vivo, the protein preferentially localizes with membrane ruffles and lamellipodia, which are active sites of actin polymerization, and modulates actin filament dynamics (36, 37). The actin regulatory functions of HSP27 are dependent on phosphorylation of the protein (7, 27, 37). Thus it is possible that agonist-dependent phosphorylation of HSP27 causes a conformational change in the protein that results in the dissociation of HSP27 from actin filaments and the release of an inhibitory constraint on the contractile apparatus. In support of this hypothesis, Bitar et al. (9) reported that incubation of permeabilized rectusigmoid SMC with monoclonal antibodies to HSP27 blocks the sustained contraction induced by bombesin and PKC. A second possibility is that activated MAPKAP kinase-2 directly phosphorylates the 20-kDa light chain subunit of myosin II, which is the on switch of actin-activated myosin ATPase activity (2). It was recently reported that MAPKAP kinase-2 is able to phosphorylate the regulatory light chain of myosin II in vitro on the same site (Ser 19) as myosin light chain kinase (31). However, the physiological relevance of this phosphorylation in vivo remains to be demonstrated. Finally, a third possibility is that p38 MAP kinase sensitizes the contractile apparatus by phosphorylating a thin filament-associated protein such as caldesmon. Caldesmon is an 87-kDa protein that binds actin and myosin and inhibits actomyosin ATPase activity (2). Caldesmon is a substrate for several protein kinases in vitro, including the ERK MAP kinases (1, 12). Phosphorylation of caldesmon by ERK1/ERK2 reduces its affinity for actin, leading to loss of inhibition of the actomyosin ATPase activity (2). These various hypotheses are currently being tested in our laboratory.

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