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Forum Review

Redox-Dependent Protein Kinase Regulation by Angiotensin II: Mechanistic Insights and Its Pathophysiology

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ABSTRACT

Reactive oxygen species (ROS) are proposed to induce cardiovascular diseases, such as atherosclerosis, hypertension, restenosis, and fibrosis, through several mechanisms. One such mechanism involves ROS acting as intracellular second messengers, which lead to induction of unique signal transductions. Angiotensin II (AngII), a potent cardiovascular pathogen, stimulates ROS production through the G protein-coupled AngII type 1 receptor expressed in its target organs, such as vascular tissues, heart, and kidney. Recent accumulating evidence indicates that through ROS production, AngII activates downstream ROS-sensitive kinases that are critical in mediating cardiovascular remodeling. Each of these ROS-sensitive kinases could potentially mediate its own specific function. In this review, we will focus our discussion on the current findings that suggest novel mechanisms of how AngII mediates activation of these redox-sensitive kinases in target organs, as well as the pathological significance of their activation. *Antioxid. Redox Signal.* 7, 1315–1326.

INTRODUCTION

ANGIOTENSIN II (AngII), the major bioactive peptide of the renin/angiotensin system, plays a critical role in controlling cardiovascular homeostasis. It is also implicated in various cardiovascular diseases, such as hypertension, atherosclerosis, restenosis after angioplasty, and heart failure (47, 75). Although accumulating evidence implicates AngII in development of these diseases, there still remains a huge void in the mechanistic insights by which AngII contributes to each of these cardiovascular diseases. This justifies the substantial research effort committed toward investigating the signal transduction network of AngII within its target organs.

There are at least two seven-transmembrane G protein-coupled receptors (GPCRs) known to mediate AngII function, namely, the AngII type 1 (AT_1) and type 2 (AT_2) receptors. These two GPCRs appear to involve quite different signal transduction pathways regarding its G protein coupling, second messengers, downstream signaling, and functions (11). We will focus mainly on the AT_1 receptor-mediated signal transduction in this review because of the following reasons. The AT_1 receptor has been shown to mediate most of the physiological, as well as pathophysiological, actions of AngII, and this subtype is predominantly expressed in cardiovascular tissues (and cells), such as the vasculature [vascular smooth muscle cells (VSMCs) and endothelial cells (ECs)], heart (cardiac myocytes and fibroblasts), and kidney (renal mesangial cells) (11, 47, 75). Through the AT_1 receptor, AngII activates a number of cytoplasmic signaling pathways (85). The AT_1 receptor interacts with various heterotrimetric G proteins, including $G_{q/11}$, G_i, G_{12}, and G_{13}, and produces classical second messengers, such as inositol trisphosphate and diacylglycerol. It also activates various intracellular protein kinases, such as receptor or nonreceptor tyrosine kinases [epidermal growth factor receptor (EGFR), platelet-derived growth factor (PDGF) receptor (PDGFR), c-Src, PYK2, focal adhesion kinase (FAK), and Janus kinase 2 (JAK2)] and serine/threonine kinases [mitogen-activated protein kinase (MAPK) family

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(extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK), p70S6 kinase, Akt/protein kinase B (PKB), and various protein kinase C (PKC) isoforms] (20, 34, 97). Importantly, AngII has been shown to induce generation of intracellular reactive oxygen species (ROS), which have been recognized as prominent players in the pathophysiology of many cardiovascular diseases (33).

ROS such as superoxide anion ($O_2 \cdot^-$) and hydrogen peroxide (H_2O_2) are produced in various cell types. In addition to the mitochondrial sources, ROS can be derived from xanthine oxidase, cyclooxygenase, lipoxygenase, nitric oxide (NO) synthase, heme oxygenese, peroxidases, hemopoietins such as heme and hematin, and NAD(P)H oxidase (35). Metabolism of ROS is tightly controlled. Dismutation of $O_2 \cdot^-$ by superoxide dismutase produces the more stable ROS, H_2O_2, which in turn is converted to water by catalase and glutathione peroxidase (35). Generation of ROS could be induced by a variety of extracellular stimuli, such as growth factors, hormones, cytokines, ultraviolet radiation, increased osmolarity, and other cellular stress. In this regard, ROS have been shown to act as novel intracellular second messengers in signal transduction pathways originating from a mix of membrane receptors (64). In turn, ROS can activate a variety of protein kinases of which many are known to promote multiple cellular responses, including growth, differentiation, apoptosis, and inflammation, in diverse tissues and cells (21, 54). Therefore, it is well appreciated that AngII could induce its pathological functions via protein kinase activation mediated by ROS production (33). However, the precise mechanisms of how these redox-sensitive kinases are activated and thereby exert their pathophysiological responses remain insufficiently characterized. In this review, we will focus our discussion on the regulation of tyrosine and serine/threonine kinases through ROS generation by AngII, as well as the functional significances of these redox-sensitive kinases activated by AngII in each target cell (and tissue).

ACTIVATION OF ROS-DEPENDENT TYROSINE AND SERINE/THREONINE KINASES BY AngII

Tyrosine kinases (receptor and nonreceptor) that are activated by AngII through ROS generation are listed in Table 1. Generally, three mechanisms have been proposed by which ROS activate a tyrosine kinase. First, ROS may directly activate kinases by altering protein–protein interactions depending on a sulfhydryl group. Second, protein tyrosine phosphatases that contain a cysteine residue in their activation site may be directly inhibited by ROS, which in turn results in tyrosine phosphorylation of the kinases and may affect their activities. Third, oxidation stimulates proteolysis of regulatory proteins that may inhibit tyrosine kinase activity (7).

Table 1. ROS-Sensitive Tyrosine Kinases Activated by AngII

Tyrosine kinase	*Tissue/cells (references)*
EGFR	VSMCs (24, 88), cardiac fibroblasts (92)
PDGFR	VSMCs (37)
JAK2	VSMCs (27, 74)
PYK2	VSMCs (23), cardiac fibroblasts (92)
Src	VSMCs (77, 87), cardiac fibroblasts (92)

Table 2 summarizes serine/threonine kinases activated by AngII in a ROS-dependent manner. These kinases are generally considered as downstream targets of the tyrosine kinases previously mentioned above. Alternatively, serine/threonine kinases could be activated directly or indirectly by ROS without participation of a tyrosine kinase. For instance, ROS may regulate a protein serine/threonine phosphatase (64). A protein serine/threonine phosphatase family member, protein phosphatase 2B, contains an Fe(II)-Zn(II) center in its active site and is inactivated by $O_2 \cdot^-$, probably as a result of oxidation of the dinuclear metal center, which may lead to serine/threonine phosphorylation and kinase activation (64). Some protein kinases are activated by H_2O_2 as a result of the oxidation of cysteine residues of upstream regulators. One example of such a scenario is the activation of apoptosis signal-regulating kinase 1 [or apoptosis-stimulated kinase 1 (ASK1)] in tumor necrosis factor (TNF) signaling. H_2O_2 generated in response to exposure of cells to TNF promotes the dissociation of thioredoxin (Trx), an inhibitory factor of ASK1, from ASK1 by oxidizing the cysteine residue of Trx (64).

ROS has also been shown to regulate G proteins directly. Nishida *et al.* showed that ROS directly activate G_i and G_o leading to ERK activation (62). In addition, Lander *et al.* showed that the Cys^{118} residue of a small G protein, Ras, is sensitive to oxidizing agents such as H_2O_2 and NO (50). The oxidation of Ras appeared to induce activation of downstream protein kinases, including ERK1/2, phosphatitidylinositol 3-kinase, and PKB (12).

Table 2. ROS-Sensitive Serine/Threonine Kinases Activated by AngII

Serine/threonine kinase	*Tissue/cells (references)*
ERK1/2	VSMCs,* ECs (94), cardiac myocytes (79), cardiac fibroblasts (92), mesangial cells (31)
ERK5/BMK1	VSMCs (86)
JNK	VSMCs (91), cardiac myocytes (51), cardiac fibroblasts (92)
p38 MAPK	VSMCs (88), ECs (10), cardiac myocytes (51), cardiac fibroblasts (92)
Akt	VSMCs (89), mesangial cells (29)
ASK1	Cardiac myocytes (39)
NIK	ECs (10)

*ERK activation has been reported to require ROS-dependent pathway (24) and -independent pathway (91).

It has been recently suggested that activation of tyrosine and serine/threonine kinase by AngII through ROS involves at least two or more distinct mechanisms in AngII target tissues or cells. The detailed mechanistic (and signaling) insights by which AngII produces ROS are described in this *Forum* issue (84). In the following sections of this review, we will discuss the role of tyrosine and serine/threonine kinases with regard to how ROS induced by AngII regulate their activation in specific target tissues/cells, together with their pathophysiological siginificances.

AngII activates receptor tyrosine kinase and serine/threonine kinase signal transduction pathways through ROS in VSMCs

It is now recognized that events such as hypertrophy, hyperplasia, and migration of VSMCs may be induced by AngII through ROS production (33). Accordingly, many researchers have focused on ROS-mediated signal transduction by AngII in VSMCs. It has been reported that AngII activates receptor tyrosine kinases such as EGFR and PDGFR, nonreceptor tyrosine kinases such as JAK2, PYK2, FAK, and Src, and serine/threonine kinases such as ERK, JNK, p38 MAPK, p70S6 kinase, and Akt/PKB through ROS production in VSMCs (20, 22, 33, 85, 88, 89, 91, 97). Several GPCR agonists including AngII can activate receptor tyrosine kinases (EGFR and PDGFR), even though these agonists do not directly activate these receptor tyrosine kinases themselves (an event referred to as "transactivation") (36). AngII induces transactivation of the EGFR, which is required for the activation of a member of the MAPK family, ERK, leading to hyperplasia and/or hypertrophy of VSMCs (15, 16, 97). ROS mediated EGFR tyrosine phosphorylation mainly at $Tyr^{1,173}$ and $Tyr^{1,068}$ induced by AngII, because antioxidants such as diphenyleneiodonium, Triton, *N*-acetylcysteine (NAC), and ebselen inhibited tyrosine phosphorylation of EGFR induced by AngII in VSMCs (24, 90). $Tyr^{1,068}$ is a major binding site for Grb2 that leads to activation of Ras/Raf/ERK1/2; on the other hand, $Tyr^{1,173}$ is a binding site for SHP-1, which negatively regulates ERK1/2. Thus, it is reasonable to speculate that EGFR transactivation through ROS not only mediates ERK activation by AngII as demonstrated (24), but also down-regulates the cascade thereafter through SHP-1 activation. We and others have shown that EGFR transactivation is required for activation of Akt/PKB, p70S6 kinase, and p38 MAPK, induction of c-Fos, and subsequent growth and migration of VSMCs (16, 18, 19, 20, 73). These data are in line with ROS-dependent Akt and p38 MAPK activation by AngII in VSMCs, whereas redox-sensitive ERK activation by AngII in VSMCs remains controversial (24, 33, 86, 88, 89, 91).

A mechanism by which ROS transactivate the EGFR in response to AngII may involve inhibition of tyrosine phosphatase, which in turn results in enhanced phosphorylation of tyrosine kinases. Supporting this notion is evidence that H_2O_2 and various other thiol-oxidizing agents could inhibit dephosphorylation of the EGFR in rat-1 cells (48). However, there appears to be an alternative pathway that ROS can utilize to transactivate the EGFR in VSMCs. Metalloprotease-dependent heparin-binding epidermal growth factor-like growth factor (HB-EGF) generation has been implicated in EGFR transactivation initiated through several GPCRs (19, 36). H_2O_2 stimulates EGFR transactivation via metalloprotease-dependent HB-EGF generation (27). Although the metalloprotease responsible for the HB-EGF generation induced by ROS has not been identified, both matrix metalloprotease (81) and a disintegrin and metalloprotease (ADAM) family of metalloproteases (4, 76) have been implicated in ectodomain shedding of HB-EGF stimulated by various agonists. Interestingly, H_2O_2 was recently shown to enhance ADAM17 activity directly and ADAM17-mediated ectodomain shedding (100). Activation of metalloprotease is thought to occur via a thiol group from a cysteine residue within the inhibitory prodomain of the metalloprotease that interacts with zinc in their catalytic domain. As ROS are known to interact with thiol groups, they may oxidize these electrophilic thiol groups and disrupt the cysteine–zinc bond, leading to activation of the metalloprotease. Thus, it is quite likely that a metalloprotease-dependent HB-EGF shedding is one of the key components of AngII-induced EGFR transactivation through ROS (20, 22). Possible signaling leading to the EGFR transactivation and its downstream cascades are illustrated in Fig. 1.

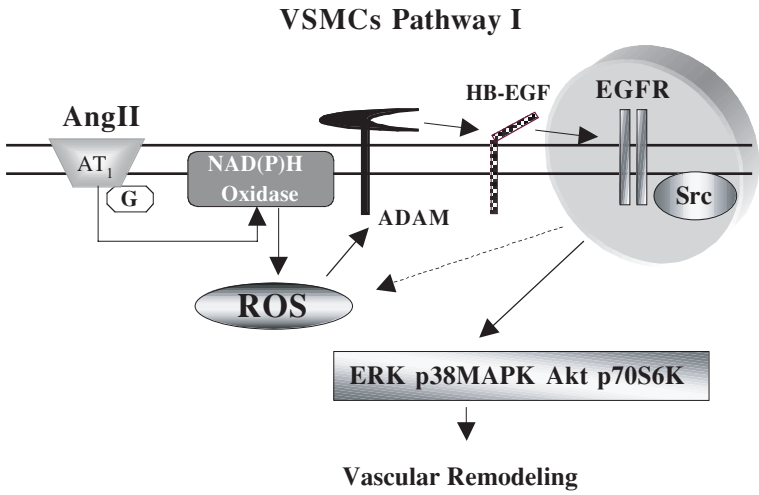

FIG. 1. Proposed signaling mechanism leading to ROS-dependent EGFR transactivation by AngII and its downstream significance in VSMCs. These cascades exist in parallel with the cascades shown in Fig. 2.

Recently, Seshiah *et al.* have shown that AngII-stimulated NAD(P)H oxidase-dependent ROS production is biphasic, with the first phase (peak at 30 s) requiring PKC activation leading to EGFR transactivation, whereas the second phase (peak at 30 min) requires EGFR transactivation and the Rac small G protein, and both phases require c-Src activation (77). It is important to note that, there are several additional mechanisms that have been shown to mediate transactivation of EGFR by AngII, such as G_q protein/phospholipase C (PLC)/Ca^{2+} pathway (15), or a G protein-independent signal involving tyrosine residue 319 phosphorylation of the AT_1 receptor (78), suggesting tissue/cell redundancy of the activation machinery.

Transactivation of EGFR has been implicated in several disease processes (4, 36), making it a current and important topic of signal transduction research. Interestingly, EGFR transactivation through GPCRs is required for cardiac hypertrophy induced by AngII, as well as by pressure overload (4, 45). Also, EGFR transactivation mediates VSMC migration in response to AngII (73). Therefore, it is now becoming clear that the transactivation of the EGFR plays a significant role in the development and progression of cardiovascular disease and that this signaling cascade may provide alternative therapeutic targets for prevention of such disease.

The PDGFR, which exists as an α or β isoform, is a transmembrane-spanning receptor tyrosine kinase (38). Similar to EGFR, the PDGFR can be activated not only by its cognate ligands, but also by other stimuli, including AngII in a ligand-independent manner (37, 72). The transactivation of the PDGFR by AngII appears to involve ROS in VSMCs (37), and the transactivation may require upstream ROS-sensing tyrosine kinases distinct from Src or JAK2 in VSMCs (37).

H_2O_2 stimulates phosphorylation of the PDGFR on tyrosine residues, one of which was identified as $Tyr^{1,021}$, a PLC-γ binding site (72). Both the binding of PLC-γ to phosphorylated $Tyr^{1,021}$ in the C-terminal tail of the PDGFR and the activation of PCL-γ are believed to be involved in cell growth and chemotaxis in certain circumstances (38). The fact that PLC-γ is recruited to PDGFR after H_2O_2 stimulation is important supportive evidence regarding H_2O_2-induced PDGFR transactivation. In addition, H_2O_2 induces the association of c-Src and PKC-δ with PDGFR. These nonreceptor kinases are required for PDGFR transactivation, but not for PDGF-BB (ligand)-induced PDGFR activation. Furthermore, it has been shown that a nonreceptor tyrosine kinase, PYK2, is required for transactivation of PDGFR, but not EGFR, in VSMCs (22).

In comparison with the EGFR, little is known about the downstream function of PDGFR transactivation. As its transactivation mechanism is not solely via autophosphorylation, the downstream significance should be different from the PDGF-mediated response in VSMCs. Our findings that PLC-γ, as well as c-Src and PKC-δ, associates with the PDGFR suggest that the receptor acts as a scaffold in ROS signaling. Interestingly, PLC-γ activated by ROS was recently shown to be involved in cell survival against ROS-induced apoptosis (54). In addition, PDGFR phosphorylation was enhanced in balloon-injured carotid arteries, but was inhibited by AT_1 receptor antagonists (1). Taken together, these data suggest the possible involvement of PDGFR transactivation in the cardiovascular remodeling process (Fig. 2).

AngII-inducible nonreceptor tyrosine kinase and serine/threonine kinase signal transduction pathway mediated by ROS in VSMCs

JAK2 is a member of the JAK family of tyrosine kinases, which are critical for signal transduction that is important for several biological functions. In particular, JAK activation is required for activation of the signal transducers and activators of transcription (STAT) pathway in response to activated cytokine receptors by cytokines (63). An early study demonstrated that AngII could also stimulate tyrosine phosphorylation and activation of JAK2, which subsequently leads to STAT isoform tyrosine phosphorylation (53). Interestingly, JAK2 activation by AngII requires ROS in VSMCs (27, 74) as indicated by the fact that H_2O_2 rapidly and strongly induces JAK2 activation in VSMCs. In addition, intracellular Ca^{2+} elevation and PKC-δ activation initiated by PLC-derived second messengers are involved in AngII-induced JAK2 activation (26). Furthermore, PYK2 is required for this JAK2 activation (26). Recently, it has been reported that the PKC-δ isoform is also required for JAK2

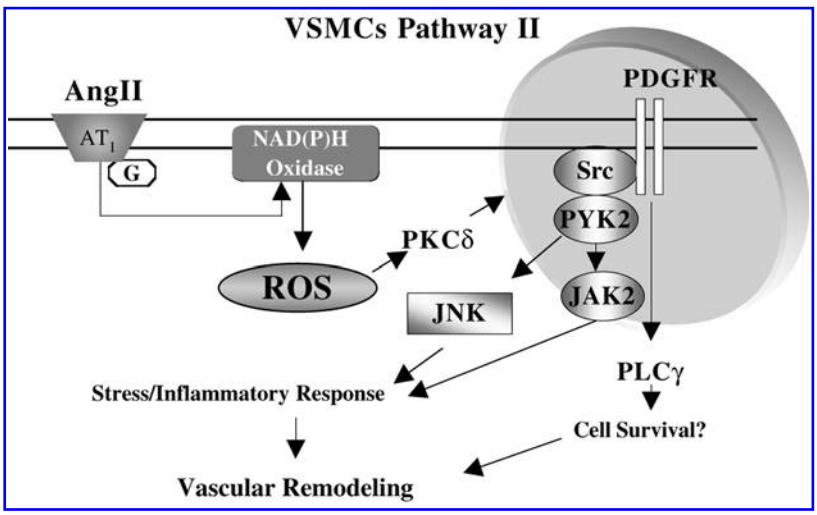

FIG. 2. Possible signaling mechanisms leading to ROS-dependent PDGFR transactivation and nonreceptor tyrosine kinase activation by AngII, and its downstream significance in VSMCs.

activation by H_2O_2 in VSMCs (27). Previously, PKC-δ has been implicated in ROS-dependent activation of other tyrosine kinases, such as c-Abl and c-Src (80). In this regard, several reports showed that H_2O_2 stimulates PKC-δ activity in various cell types, including VSMCs (27). Utilizing dominant-negative PYK2 mutants, it has been shown that PYK2, which is also downstream of PKC-δ in VSMCs, is required for JAK2 activation, but not for EGFR activation by H_2O_2 (27). Taken together, these findings suggest that at least two major tyrosine kinase activation mechanisms are utilized by ROS in VSMCs. One mechanism involves ROS-activated PKC-δ that leads to the activation of PYK2/JAK2 pathway or the PDGFR transactivation (Fig. 2). The other mechanism involves activation of ROS-dependent metalloprotease cleavage of proHB-EGF to generate active HB-EGF, which leads to EGFR transactivation shown in the previous Fig. 1.

PYK2 is a nonreceptor tyrosine kinase, also identified as cell adhesion kinase β, or related adhesion focal tyrosine kinase (5). Generally, PYK2 requires Ca^{2+} and/or PKC for its activation by GPCR agonists. Also, PYK2 can be activated by a wide variety of extracellular stimuli, such as GPCR agonists, growth factors, cytokines, and environmental stress (5). In VSMCs, AngII rapidly stimulates PYK2 kinase activity (25, 69) and phosphorylation at Tyr^{402}, a putative autophosphorylation site of PYK2 (23, 25). In VSMCs, PYK2 is also activated by extracellular administration of H_2O_2 (23). AngII stimulates association of PYK2 with Src and induces JNK activation through PYK2 in VSMCs (17, 25). Interestingly, c-Src mediates ROS-dependent JNK activation, but not ERK and p38 MAPK in VSMCs (98). Based on these findings in VSMCs, it is likely that a ROS-sensitive kinase, PYK2, plays a major role in mediating JNK and the JAK/STAT pathway activation leading to stress and inflammatory responses.

Src family kinases now include nine members, of which Src, Fyn, and Yes are expressed in most tissues. These kinases can be activated by a variety of receptors, channels, and extracellular stress, including ROS (82). As mentioned above, Src kinases seem to be critically involved in ROS-mediated activation of other tyrosine kinases, such as activation of EGFR, PDGFR, and JAK2 in VSMCs, whereas c-Src function may be required for ROS generation by AngII in VSMCs as well as (77, 87). Importantly, Src was previously shown to mediate the phosphorylation of paxillin, which is responsible for focal adhesion formation in VSMCs (42). Src kinase activation by ROS likely requires an interaction with PKC-δ. H_2O_2 induces phosphorylation of c-Src at Tyr^{418}, a critical site for activation, leading to association of c-Src with PKC-δ in response to H_2O_2 (72). These findings further suggest that interaction of PKC-δ with other nonreceptor tyrosine kinases leads to the phosphorylation of each kinase by the other, initiating ROS-dependent signal transduction.

A previous article mentioned a role of ROS and ceramide in nuclear factor-κB (NFκB) activation through AT_2 receptor in VSMCs (68). However, involvement of possible redox-sensitive kinases in this cascade remains unknown.

Role of ROS and ROS-sensitive kinase activation induced by AngII in ECs

The vascular endothelium regulates local hemodynamics, as well as vascular cell adhesion, by the production and release of multiple humoral factors (59). One critical mediator of endothelial function is NO, which under normal physiological conditions provides local antithrombotic actions and regulation of vasomotor tone (59). The actions of NO can be altered severely under conditions of oxidative stress. Of particular importance is the avid interaction of NO with $O_2\cdot^-$, which results in the formation of the highly aggressive reactive nitrogen species, peroxynitrite ($ONOO^-$) (6). Relative to other biological oxidants, $ONOO^-$ possesses a high affinity to nitrate tyrosine residues, both protein-bound and free, forming 3-nitrotyrosine (3NT) (6). Therefore, in addition to the loss of important NO-mediated signaling events, oxidative destruction of NO can have direct cytotoxic actions as well. Elevations in both protein and free 3NT (plasma, cerebrospinal fluid) are relevant phenomena in human cardiovascular disease (49). However, the critical stimuli inducing these events, the putative cellular targets affected, and the cellular defense mechanisms to accommodate these changes remain incompletely defined. Although AngII is a well described producer of ROS in vascular smooth muscle, the mechanisms by which AngII modulates endothelial function remain poorly understood.

In addition, ROS causes a variety of adverse biological effects in ECs, including the production of inflammatory mediators (52), expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) (65), increased cell permeability (46), and cell proliferation and migration (95). AngII induces $O_2\cdot^-$ generation and NAD(P)H oxidase subunit gp91phox mRNA and protein expression in human umbilical vein ECs (67) and p22phox mRNA expression in cardiac microvascular ECs (94). The stimulation of NO production by AngII is thought to be a vascular protection mechanism (96). Cai *et al.* have shown that endogenous H_2O_2, derived from NAD(P)H oxidase, mediates endothelial NO production in response to AngII (8). Thus, under disease conditions associated with elevated levels of AngII, this response may represent a compensatory mechanism. On the other hand, Mihm *et al.* showed that AngII induced EC $ONOO^-$ formation (57). Moreover, an *in vivo* experiment showed that AngII infusion decreased NO production in aorta (58). Specifically, endothelial NO synthase (eNOS) may become uncoupled by AngII infusion, causing $O_2\cdot^-$ production rather than NO production (58, 96). Based on these findings, the balance between generation of ROS, NO, and $ONOO^-$ in ECs and VSMCs could be an important determinant of the pathological AngII function in mediating endothelial (vessel) dysfunction.

In addition, AngII can activate ERK through ROS in cardiac microvascular ECs (94) and ROS-sensitive ERK activation by AngII induced endothelin-1 (ET-1) mRNA expression through activator protein-1 (AP-1) activation (40). As ET-1 is a strong vasoconstrictor and inducer of VSMC growth, this cascade plays an important role in cardiovascular disease and vascular remodeling (66). Furthermore, AngII induces NFκB-dependent transcription mediated by NFκB-inducing kinase (NIK) in ECs, leading to the up-regulation of ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) expression, and this cascade is also involved in ROS and p38 MAPK activation (10). The role of ROS and that involving ROS-sensing kinases in endothelial function mediated by AngII are illustrated in Fig. 3.

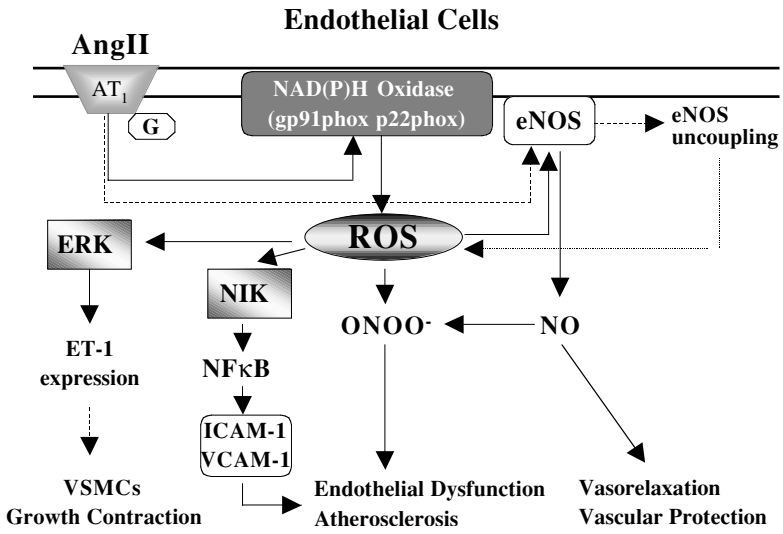

FIG. 3. The role of ROS and ROS-sensing kinases in endothelial function regulated by AngII.

AngII-inducible protein kinase signaling pathway mediated by ROS in cardiac myocytes and fibroblasts

In cardiac myocytes, it is suggested that hypertrophic effects of AngII are mediated through ROS generation (70). To support this notion, AngII induced ROS generation and hypertrophy in cardiac myocytes, both of which were inhibited by antioxidants (61). AngII induces β-myosin heavy chain gene expression in cardiac myocytes through the Ras/Raf/ERK pathway, and antioxidants such as NAC and catalase decrease ERK phosphorylation and inhibit β-myosin heavy chain promoter activity induced by AngII (79). Recently, Liu *et al.* showed that AngII induced JNK and p38 MAPK activation in addition to ERK, and that antioxidants inhibited these MAPK activations, as well as AP-1 and NFκB reporter activities induced by AngII in cardiac myocytes (51). ASK1, redox-sensitive mitogen-activated protein kinase kinase kinase (MAPKKK), existing upstream of JNK and p38 MAPK (41), seems to play a key role in these cascades. Hirotani *et al.* showed that in neonatal rat ventricular myocytes, NAC inhibited activation of ASK1 induced by AngII, and dominant-negative ASK1 transfection attenuated NFκB activation and the myocyte hypertrophy induced by AngII (39). This evidence is further supported by recent *in vivo* findings that AngII induces activation of ASK1, p38 MAPK, and JNK, in mouse left ventricle through ROS generation (43). Moreover, in ASK knockout mice, activation of p38 MAPK and JNK, as well as cardiac hypertrophy and remodeling, induced by AngII was attenuated (43). In addition, AngII has been shown to activate JAK2 (55) and EGFR tyrosine kinases (4, 83) in cultured neonatal cardiac myocytes. Both kinases are implicated in AngII-induced cardiac remodeling (4, 55, 83), and were shown to be activated by AngII through ROS-dependent mechanisms in other cell types, such as VSMCs. Also, ROS have been shown to induce cardiac remodeling through the Ras/ERK cascade, which is dependent on Src kinase activity in cardiac myocytes (2). Src kinase activation by AngII mediates cardiac hypertrophy (71). Therefore, the possible involvement of these protein tyrosine kinases in regulation of the ROS-dependent signal transduction pathway of AngII in cardiac myocytes should be considered. The proposed ROS-sensitive cascades from AngII to cardiac remodeling are illustrated in Fig. 4. Because a number of other signaling pathways, such as the calcineurin/NFAT (nuclear factors of activated T cells) pathway, are also implicated in cardiac remodeling, further studies are obviously necessary to connect and/or separate ROS-sensitive and ROS-insensitive signal transduction cascades leading to cardiac remodeling.

Cardiac fibroblasts play an important role in maintaining cardiac function by providing structural support for cardiomyocytes and serving as a source for paracrine growth factors (32). After myocardial infarction, reactive fibrosis results in excess scar formation as proliferating fibroblasts invade the necrotic area. This remodeling leads to an increase of the ventricular stiffness and ultimately compromises the function of the heart (13). AngII induces proliferation of cardiac fibroblasts, which involves activation of many protein kinases (14). Accordingly, the AngII-induced signal transduction pathway in cardiac fibroblasts could contribute to cardiovascular disease involving fibroblast dysfunction/remodeling.

Wang *et al.* showed that NAC inhibited ERK, JNK, and p38 MAPK activation induced by AngII in cardiac fibroblasts (92). In addition, NAC inhibited EGFR transactivation, tyrosine phosphorylation of Src and PYK2, and the Src-PYK2 complex formation induced by AngII in cardiac fibroblasts (93). Recently, it has been shown that ERK/AP-1-mediated transcription stimulated by ROS is required for AngII-induced proliferation and ET-1 gene expression in cardiac fibroblasts (9). As ET-1 is believed to be involved in AngII-induced cardiac remodeling (66), paracrine production of ET-1 from fibroblast could participate in cardiac remodeling as illustrated in Fig. 5.

Redox-sensitive protein kinase activation induced by AngII in mesangial cells

In addition to vessels and heart, AngII activates signal transduction pathways in other tissues expressing AT_1, such as the kidney, which may be the cause of diseases such as

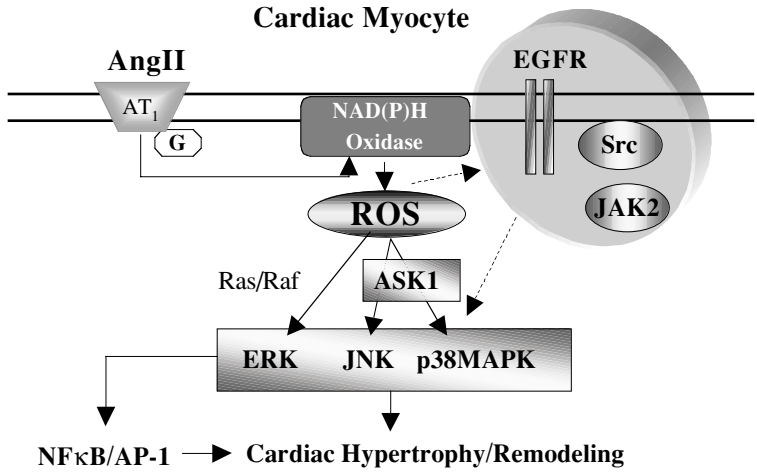

FIG. 4. Proposed ROS-sensitive cascades involved in tyrosine and serine/threonine kinase activation by AngII in cardiac myocytes.

renal sclerosis and fibrosis (56). AngII stimulates proliferation of mesangial cells and contributes to the pathogenesis of fibrosis of the glomerular microvascular bed (3, 56). Renal mesangial cells possess many mitogenic GPCRs, including AngII. In addition to these GPCRs, mesangial cells also express receptor tyrosine kinases, which may participate in the proliferative phase of chronic renal failure or in the recovery from renal damage. AngII modulates the glomerular filtration rate via contraction of mesangial cells, and stimulates mesangial cell growth and hypertrophy. Interestingly, AngII induces $O_2^{\cdot -}$ generation and antioxidants inhibit hypertrophy induced by AngII in mesangial cells (44). Gorin *et al.* showed that AngII induced protein synthesis and hypertrophy through an arachidonic acid/redox-dependent pathway and through Akt/PKB activation independent of phosphoinositide 3-kinase in mesangial cells (29). Furthermore, the same group of researchers showed that arachidonic acid activates Rac1/Nox4-based NAD(P)H oxidase and induces subsequent generation of ROS, which mediate Akt/PKB (30) and ERK (31) activation and protein synthesis in mesangial cells stimulated with AngII. In addition, AngII induces PDGFR transactivation (60), and ROS induce tyrosine phosphorylation of PDGFR (28) and FAK (99) in mesangial cells. Based on these findings, it is possible that PDGFR and FAK may be involved in the AngII/ROS signaling pathway in mesangial cells. The proposed ROS-sensitive cascades from AngII to hypertrophy in mesangial cells are illustrated in Fig. 6.

FUTURE DIRECTION AND PERSPECTIVE

In summary, the findings discussed here clearly support the theory that AngII activates tyrosine kinases and serine/threonine kinases by ROS through distinct mechanisms in AngII target tissues or cells, and that each ROS-sensitive kinase has a unique role in mediating cardiovascular diseases. However, there is still a considerable amount of information to reveal regarding the detailed mechanism of AngII/ROS signaling pathways. In VSMCs, there are two distinct and very significant contributors, a metalloprotease and

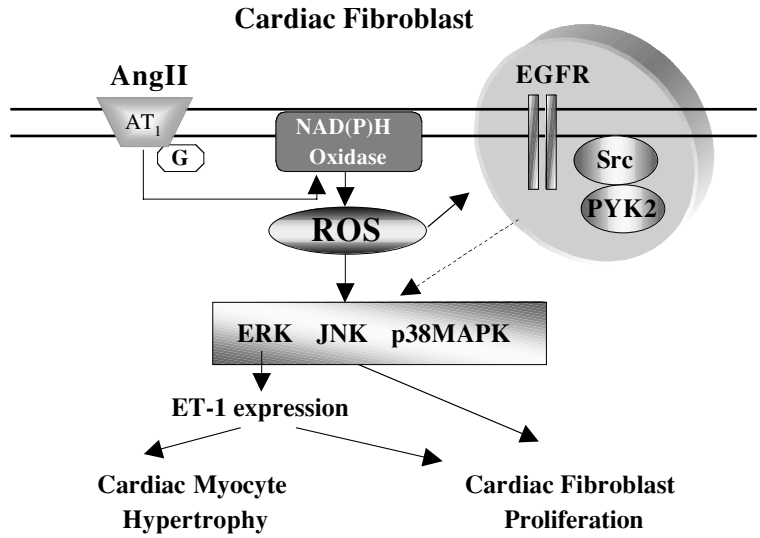

FIG. 5. Possible signaling mechanisms leading to ROS-sensitive kinase activation in cardiac fibroblasts.

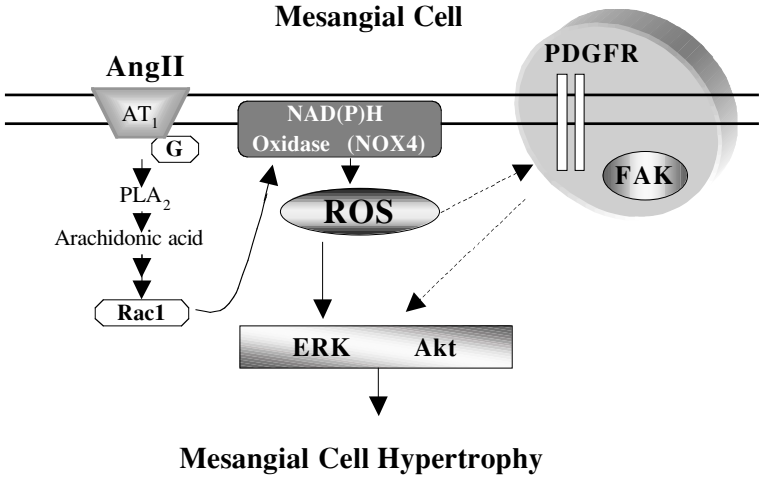

FIG. 6. Proposed ROS-sensitive kinase cascades activated by AngII in mesangial cells.

PKC-δ. However, further extensive research is required to determine the detailed activation mechanisms of these factors. In ECs, cardiac myocytes, and other cells, some ROS-dependent serine/threonine kinases and downstream targets induced by AngII were reported; however, there is not yet enough information regarding tyrosine kinase pathways induced by AngII through ROS. Moreover, additional *in vivo* experiments to analyze the detailed pathophysiological significance of each redox-sensitive kinase activated by AngII will be needed. Further characterization and understanding of the cellular mechanisms, as well as the pathophysiological significances, involving ROS-dependent protein kinase activation by AngII will provide new targets for effective therapies toward cardiovascular diseases.

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ABBREVIATIONS

ADAM, a disintegrin and metalloprotease; AngII, angiotensin II; AP-1, activator protein-1; ASK1, apoptosis-stimulated kinase 1; AT_1, angiotensin II type 1 receptor; AT_2, angiotensin II type 2 receptor; ECs, endothelial cells; EGFR, epidermal growth factor receptor; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; ET-1, endothelin-1; FAK, focal adhesion kinase; GPCRs, G protein-coupled receptors; HB-EGF, heparin-binding epidermal growth factor-like growth factor; H_2O_2, hydrogen peroxide; ICAM-1, intercellular adhesion molecule-1; JAK2, Janus kinase 2; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NAC, *N*-acetylcysteine; NAD(P)H, nicotinamide adenine dinucleotide phosphate in the reduced form; NFκB, nuclear factor-κB; NIK, NFκB-inducing kinase; NO, nitric oxide; 3NT, 3-nitrotyrosine; $O_2\cdot^-$, superoxide anion; $ONOO^-$, peroxynitrite; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PKB, protein kinase B; PKC, protein kinase C; PLC, phospholipase C; ROS, reactive oxygen species; STAT, signal transducers and activators of transcription; TNF, tumor necrosis factor; Trx, thioredoxin; VCAM-1, vascular cell adhesion molecule-1; VSMCs, vascular smooth muscle cells.

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