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Reactive oxygen species and angiotensin II signaling in vascular cells - implications in cardiovascular disease

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Abstract

Diseases such as hypertension, atherosclerosis, hyperlipidemia, and diabetes are associated with vascular functional and structural changes including endothelial dysfunction, altered contractility and vascular remodeling. Cellular events underlying these processes involve changes in vascular smooth muscle cell (VSMC) growth, apoptosis/anoikis, cell migration, inflammation, and fibrosis. Many factors influence cellular changes, of which angiotensin II (Ang II) appears to be amongst the most important. The physiological and pathophysiological actions of Ang II are mediated primarily via the Ang II type 1 receptor. Growing evidence indicates that Ang II induces its pleiotropic vascular effects through NADPH-driven generation of reactive oxygen species (ROS). ROS function as important intracellular and intercellular second messengers to modulate many downstream signaling molecules, such as protein tyrosine phosphatases, protein tyrosine kinases, transcription factors, mitogen-activated protein kinases, and ion channels. Induction of these signaling cascades leads to VSMC growth and migration, regulation of endothelial function, expression of pro-inflammatory mediators, and modification of extracellular matrix. In addition, ROS increase intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), a major determinant of vascular reactivity. ROS influence signaling molecules by altering the intracellular redox state and by oxidative modification of proteins. In physiological conditions, these events play an important role in maintaining vascular function and integrity. Under pathological conditions ROS contribute to vascular dysfunction and remodeling through oxidative damage. The present review focuses on the biology of ROS in Ang II signaling in vascular cells and discusses how oxidative stress contributes to vascular damage in cardiovascular disease.

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Introduction

Angiotensin II (Ang II), originally described as a potent vasoconstrictor, is now recognized as a multifunctional hormone influencing many cellular processes important in the regulation of vascular function, including cell growth, apoptosis, migration, inflammation, and fibrosis (1,2). Ang II is an important growth modulator of blood vessels and renal organogenesis during development and plays a critical role in regulating

blood pressure and fluid homeostasis in physiological conditions. In pathological conditions, through its vasoconstrictor, mitogenic, pro-inflammatory, and pro-fibrotic actions, Ang II contributes to altered vascular tone, endothelial dysfunction, structural remodeling, and vascular inflammation, characteristic features of vascular damage in hypertension, atherosclerosis, vasculitis, and diabetes (2-5).

The subcellular mechanisms and signaling pathways whereby Ang II mediates its physiological and pathophysiological vascular effects are complex (2). Growing evidence indicates that production of reactive oxygen species (ROS) and activation of reduction-oxidation (redox)-dependent signaling cascades are critically and centrally involved in Ang II-induced actions (3,5). All vascular cell types, including endothelial cells, smooth muscle cells, adventitial fibroblasts, and resident macrophages, produce ROS (6-10). Of particular importance in the vasculature are superoxide ($\bullet O_2^-$) and hydrogen peroxide (H_2O_2), since these ROS act as inter- and intra-cellular signaling molecules. The major source of ROS in the vascular wall is non-phagocytic NADPH oxidase, which is regulated by vasoactive agents (Ang II, ET-1, thrombin, serotonin), cytokines (IL-1, TNFα), growth factors (PDGF, IGF-1, EGF) and mechanical forces (cyclic stretch, laminar and oscillatory shear stress) (5). The best characterized system in vascular cells is Ang II-stimulated NADPH oxidase-mediated generation of $\bullet O_2^-$, which appears to be upregulated in hypertension, atherosclerosis and diabetes (5).

The present review focuses on recent progress in mechanisms whereby Ang II generates ROS in vascular cells, how ROS influence signaling events and cellular function and what the implications are in vascular function and remodeling in cardiovascular diseases. Emerging concepts on mechanisms of signal transduction by ROS that involve perturbations in cellular redox state and oxidative modifications of proteins are also discussed.

Reactive oxygen species, redox signaling and oxidative stress

ROS are formed as intermediates in redox processes, leading from oxygen to water (11). The univalent reduction of oxygen, in the presence of a free electron (e), yields $\bullet O_2^-$, H_2O_2 and •OH (Figure 1). Superoxide has an unpaired electron, which imparts high reactivity and renders it unstable and short-lived. It is water soluble and membrane impermeable, but can cross cell membranes via anion channels (12,13). In physiological conditions in aqueous solutions at a neutral pH, $\bullet O_2^-$ dismutates yielding H_2O_2. However, when produced in excess, a significant amount of $\bullet O_2^-$ reacts with NO to produce $ONOO^-$ (14).

Hydrogen peroxide is produced mainly from dismutation of $\bullet O_2^-$. This reaction can be spontaneous or can be catalyzed by superoxide dismutase (SOD), of which there are three isoforms, CuZnSOD, MnSOD and extracellular SOD (EC-SOD) (11). The SOD-catalyzed dismutation is favored when the concentration of $\bullet O_2^-$ is low and when the concentration of SOD is high, which occurs under physiological conditions. Unlike $\bullet O_2^-$, H_2O_2 is not a free radical and is a much more stable molecule. Hydrogen peroxide is lipid soluble, crosses cell membranes and has a longer half-life than $\bullet O_2^-$. In biological systems, it is scavenged by catalase and by glutathione peroxidase (13). Hydrogen peroxide can also be reduced to generate the highly reactive •OH in the presence of metal-containing molecules such as Fe^{2+} (11). Hydroxyl radical is extremely reactive and, unlike $\bullet O_2^-$ and H_2O_2, which travel some distance from their site of generation, •OH induces local damage where it is formed. In the vasculature, $\bullet O_2^-$, H_2O_2, NO, $OONO^-$, and •OH are all produced to varying degrees. These pro-oxidants are tightly regulated by anti-oxidants such as SOD, catalase, thioredoxin, glutathione, anti-oxidant vitamins, and other small molecules (15,16). Under normal

conditions, the rate of ROS production is balanced by the rate of elimination.

ROS share several features with classical second messengers and have been implicated as important signaling molecules. Similar to second messengers, production of ROS is tightly regulated by extracellular stimuli. ROS are small molecules that can diffuse locally, their existence is transient and they act on specific downstream effectors to influence cell activity and function (17). Redox signaling involves at least one reaction in which oxidation of a signaling molecule by a ROS occurs and which is reversible (18). Physiologic generation of ROS has been implicated in a variety of biological responses from transcriptional activation to cell proliferation. "Redox regulation" refers to the biological responses maintaining cell homeostasis against oxidative excess. Under pathological conditions, a disequilibrium between ROS generation and antioxidant protection results in increased bioavailability of ROS leading to a state of oxidative stress (19,20). Hence oxidative events in which ROS play specific roles in signaling cascades and which are non-damaging are referred to as redox-signaling processes (17,18). On the other hand, an oxidative burden in which ROS cause injury and where repair or cell death are non-specific responses with respect to the involvement of oxidants is termed oxidative stress. The pathogenic outcome of oxidative stress is oxidative damage (13), a major cause of vascular injury in cardiovascular disease.

Ang II-induced production of reactive oxygen species in vascular cells

Vascular NADPH oxidase

Ang II elicits its actions via two distinct receptors, the Ang II type 1 (AT_1) and Ang II type 2 receptors (AT_2) (2). Most known physiological and pathophysiological effects of Ang II are mediated via AT_1 receptors, which couple to multiple interacting signal transduction cascades, leading to diverse biological actions. These signaling processes are multiphasic with distinct temporal characteristics and have been well described in recent reviews (2,21).

Exciting new research in the field of vascular biology is the demonstration that AT_1 receptor activation stimulates non-phagocytic NADPH oxidase and generation of $\bullet O_2^-$ in various vascular cell types, including vascular smooth muscle cells (VSMC) (5,6), endothelial cells (7) and fibroblasts (8). Vascular NADPH oxidase is similar, but not identical, to neutrophil NADPH oxidase, as summarized in Table 1. The prototypical phagocytic NADPH oxidases are multimeric protein complexes comprising membrane-bound

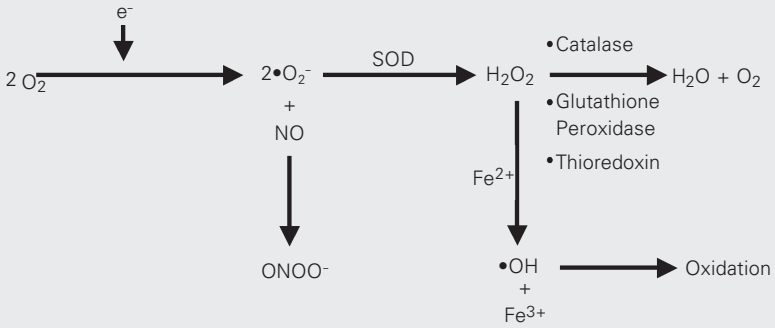

Figure 1. Diagram demonstrating how the univalent reduction of oxygen, in the presence of a free electron (e), yields $\bullet O_2^-$, H_2O_2 and •OH. SOD = superoxide dismutase.

Table 1. Characteristics of neutrophil and vascular NADPH oxidase.

Characteristic	Neutrophil	Vascular
Activity in basal state	Inactive	Constitutively active
Inducible by	Cytokines, pathogens	Vasoactive agents, growth factors, cytokines, physical factors
Nox2 homologues	Nox2	Nox1/Nox2/Nox4/Nox5
Kinetics of $\bullet O_2^-$ release	Burst-like	Slow and sustained
$\bullet O_2^-$ concentration	High	Low
Site of $\bullet O_2^-$ generation	Extracellular	Intracellular
Substrate	NADPH	NADH/NADPH
Small G-protein	Rac2	Rac1

flavocytochrome b558 (formed by gp91phox (Nox2) and p22phox), up to three cytoplasmic subunits, p47phox, p67phox and p40phox and a regulatory G-protein (Rac1 or Rac2) (9). All neutrophil subunits have been demonstrated, to varying degrees, in vascular cells (6-9) (Figure 2). In addition, Nox2 homologues, Nox1, a 563-amino acid protein that shares 55% homology with gp91phox, and Nox4, a 578-amino acid protein with 39% homology to gp91phox, have been implicated to play a role in vascular cell $\bullet O_2^-$ production (22,23). Both Nox1 and Nox4 are expressed in vascular cells and are regulated by factors that stimulate ROS generation, such as Ang II and PDGF (24,25). Nox1 was initially suggested to be a subunit-independent low capacity $\bullet O_2^-$ generating enzyme involved in the regulation of mitogenesis (22). However, recent data indicate that Nox1 requires p47phox and p67phox and that it is regulated by NoxO1 (Nox organizer 1, a p47phox homologue) and NoxA1 (Nox activator 1, a p67phox homologue) (26). Nox4 has recently been implicated to be the major catalytic component in endothelial cells (27). Although the renin-angiotensin system has been demonstrated to upregulate vascular Nox1 and Nox4 *in vitro* and *in vivo* (28), the physiological significance of these processes in the cardiovascular system awaits clarification.

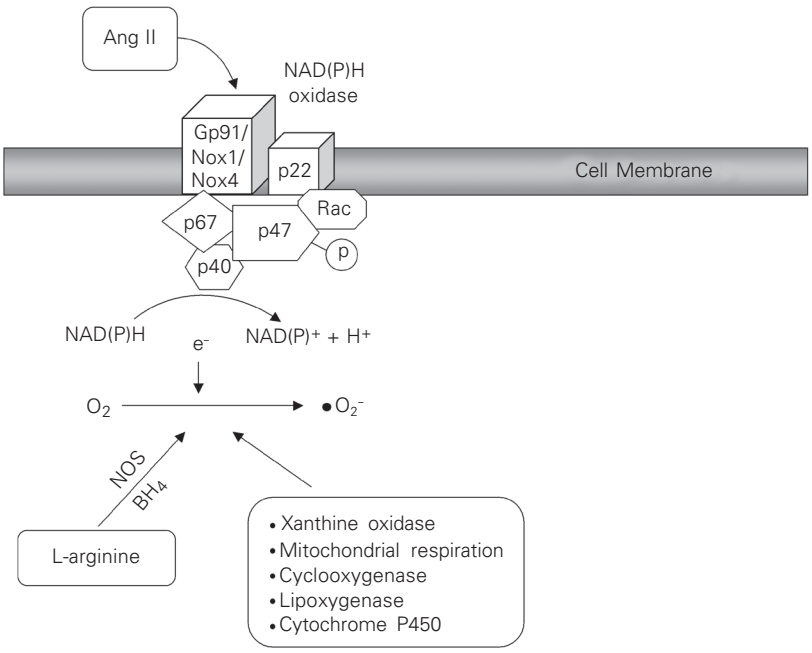

Figure 2. Generation of O_2^- and H_2O_2 from O_2 in vascular cells. Many enzyme systems, including NADPH oxidase, xanthine oxidase and uncoupled nitric oxide synthase (NOS) among others, have the potential to generate reactive oxygen species.

Activation of NADPH oxidase is a multistep process initiated by serine phosphorylation of p47phox, which triggers complex formation of cytoplasmic subunits followed by translocation to the membrane where, together with Rac, it associates with cytochrome b558 to assemble the active oxidase (9) (Figure 2). Of the many vasoactive factors that stimulate this process, Ang II appears to be one of the most important in the vasculature (5,6,29). Mechanisms linking Ang II to the enzyme and upstream signaling molecules modulating NADPH oxidase in VSMCs have not been fully elucidated, but PLD, PKC, c-Src, EGFR transactivation, PI3K, and Rac may be involved (30-32). In its activated state, NADPH oxidase accepts electrons from its substrate NADPH and donates these to molecular oxygen. In this way, a one-electron reduction of oxygen to $\bullet O_2^-$ is catalyzed at the expense of NADPH according to the following reaction: $2O_2 + NADPH - NADPH\ oxidase \rightarrow 2\bullet O_2^- + NADP^+ + H^+$.

Other enzymatic sources

Nitric oxide synthase (NOS), the enzyme primarily responsible for NO production, can also generate $\bullet O_2^-$ in conditions of substrate (arginine) or co-factor (tetrahydrobiopterin) (BH_4) deficiency (33). These findings have led to the concept of "NOS uncoupling", where the activity of the enzyme for NO production is decreased in association with an increase in NOS-dependent $\bullet O_2^-$ formation. Ang II may play a role in these processes in pathological conditions (34). eNOS uncoupling has been demonstrated in atherosclerosis (35), diabetes (36), hyperho-

mocystinemia (37), and hypertension (38), all of which are associated with activation of the renin-angiotensin system. Other enzymatic sources capable of generating ROS in the vasculature are xanthine oxidase, cytochrome P450, mitochondrial respiratory chain enzymes, and phagocyte-derived myeloperoxidase (4-6). However, the contribution of these enzymes to vascular generation of ROS is relatively minor compared with NADPH oxidase.

Signaling molecules targeted by reactive oxygen species

Observations that ROS could function as second messengers were first made in the 1970s when it was demonstrated that exogenous H_2O_2 mimics the action of insulin and that insulin and growth factors stimulate cellular H_2O_2 production (39). Accumulating evidence indicates that endogenous ROS participate in signaling cascades in many cell types (17,18).

ROS appear to be important participants in Ang II signaling in vascular cells. This is based on the findings that 1) Ang II is capable of generating ROS in vascular cells, 2) antioxidants and inhibitors of ROS-generating systems abolish agonist-mediated signaling pathways, and 3) exogenous addition of oxidants activate the same signaling cascades as Ang II. Major targets of ROS include transcription factors, protein tyrosine phosphatases (PTP), protein tyrosine kinases (PTK), mitogen-activated protein (MAP) kinases, ion channels, phospholipases, and transcription factors (40), all of which are regulated by Ang II (Figure 3).

Transcription factors

Transcription factors were the first signaling proteins identified as redox-sensitive. The DNA binding activity is regulated through specific cysteine motifs that need to be reduced for activity. Nuclear factor κB (NFκB), which is activated by Ang II in vascular cells, is the prototype of redox-sensitive transcription factors. NFκB is sequestered in the cytoplasm in a complex with its inhibitor IκB. ROS influence NFκB activity by oxidative modification of cysteine residues, by IκB degradation and by oxidative enhancement of upstream signal cascades (40). NFκB regulates transcription of many genes involved in vascular inflammation and growth, including interleukins, adhesion molecules and proto-oncogenes (41). Other Ang II-activated redox-sensitive transcription factors include activator protein-1 (AP-1) and hypoxia-inducible factor-1 (HIF-1). AP-1 is a transcription factor complex formed by homo- or heterodimerization of members of the c-Jun and c-Fos families of proteins and influences vascular cell differentiation and growth. ROS regulate AP-1 activity through numerous mechanisms and targets, including the reversible S-glutathi-

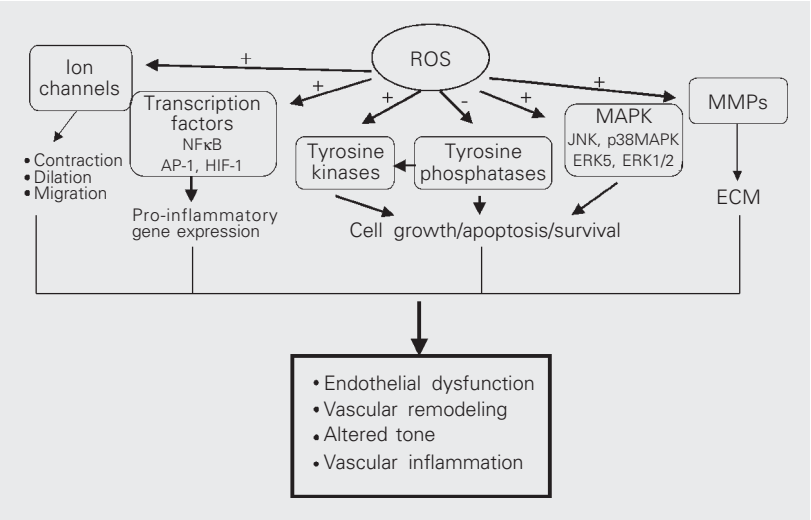

Figure 3. Redox-dependent signaling pathways by Ang II in vascular smooth muscle cells. Intracellular reactive oxygen species (ROS) modify the activity of tyrosine kinases, such as Src, Ras, JAK2, Pyk2, PI3K, and EGFR, as well as mitogen-activated protein kinases (MAPK), particularly p38MAPK, JNK and ERK5. ROS may inhibit protein tyrosine phosphatase activity, further contributing to protein tyrosine kinase activation. ROS also influence gene and protein expression by activating transcription factors, such as NFκB, activator protein-1 (AP-1) and hypoxia-inducible factor-1 (HIF-1). ROS stimulate ion channels, such as plasma membrane Ca^{2+} and K^+ channels, leading to changes in cation concentration. Activation of these redox-sensitive pathways results in numerous cellular responses which, if uncontrolled, could contribute to hypertensive vascular damage. -, inhibitory effect; +, stimulatory effect; ECM, extracellular matrix; MMPs, matrix metalloproteinases.

olation of a single conserved cysteine residue, the reversible redox regulation by thioredoxin and the nuclear protein Ref1 (42) and through regulation by the c-Jun N-terminal kinase (JNK) cascade. JNK phosphorylates serine residues 63 and 73 of the NH_2-terminal transactivation of c-Jun, required for functional activation of AP-1 (43).

Protein tyrosine phosphatases and protein tyrosine kinases

Currently the best-established direct molecular targets of ROS are PTPs. Protein-tyrosine phosphorylation is a major mechanism for post-translational modification of proteins and plays a critical role in regulating cell proliferation, differentiation, migration, and transformation. The level of tyrosine phosphorylation in cells is controlled by the tightly regulated balance between PTK and PTPs (44). By dephosphorylating PTK substrate proteins, PTPs counteract effects of PTK activity. Hence PTPs may be considered as negative regulators and terminators of a signaling process initiated by PTK activation. Exposure of cells to low doses of oxidants or thiol-directed agents induces an increase in tyrosine phosphorylation due to PTP inactivation.

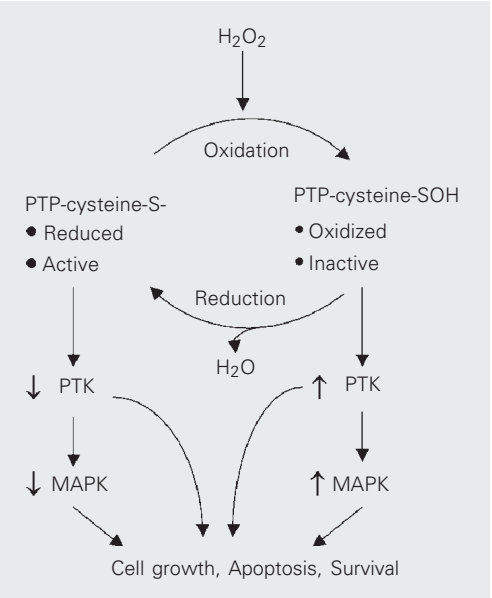

Figure 4. Protein tyrosine phosphatases (PTPs) are susceptible to oxidation and inactivation by reactive oxygen species (ROS). All PTPs possess a redox-regulated cysteine, which catalyzes the hydrolysis of protein phosphotyrosine residues by the formation of a cysteinyl-phosphate intermediate. Oxidation of this cysteine residue to sulfenic acid by H_2O_2 renders the PTP completely inactive. Since the oxidation of PTP is reversible, PTPs exist in two forms: an active state with a reduced cysteine or an inactive state with an oxidized cysteine. Inactivation of PTP is associated with increased activation of protein tyrosine kinases (PTK) and mitogen-activated protein kinases (MAPK).

Protein tyrosine phosphatases. PTPs are a large, structurally diverse family of receptor and non-receptor enzymes that are critical regulators of multiple signaling pathways (44). Because of their particular structure, PTPs are susceptible to oxidation and inactivation by ROS. All PTPs possess a conserved 230-amino acid domain that contains a reactive and redox-regulated cysteine, which catalyzes the hydrolysis of protein phosphotyrosine residues by the formation of a cysteinyl-phosphate intermediate (45). This cysteine forms thiol phosphate, an intermediate in the dephosphorylation reaction of PTPs. Oxidation of this cysteine residue to sulfenic acid by H_2O_2 renders the PTP completely inactive (45) (Figure 4). Since the oxidation of PTP is reversible, PTPs exist in two forms: an active state with a reduced cysteine or an inactive state with an oxidized cysteine. Activation and inactivation of PTPs are regulated by extracellular signals, including Ang II (46) and EGF and H_2O_2 plays a major role as a secondary messenger in this process (45) (Figure 4). Lee and colleagues (47) demonstrated that EGF-induced PTP1B inactivation is dependent on reversible oxidation of cysteine residues by H_2O_2. Recent studies suggest that PTP1B may be more efficiently regulated by $\bullet O_2^-$ than by H_2O_2 (48). Peroxynitrite rapidly and irreversibly inhibits PTPs, supporting the role of this ROS in oxidative damage.

Besides soluble phosphatases, receptor PTP (RPTP) can also be modulated by oxidative stress (49). A model has been proposed in which oxidative stress induces a conformational change in RPTPα-D2, leading to stabilization of RPTPα dimers, and thus to inhibition of RPTPα activity (49). In addition, the inactivation of PTPs is involved in oxidative stress-induced activation of several PTK such as the EGFR, insulin receptor, Lck and Fyn (41). This is particularly important with respect to Ang II, which mediates many of its signaling events in vascular cells through EGFR trans-

activation (2). H_2O_2 has also been shown to regulate MAP kinases through inhibition of PTP activity of CD45, SHP-1 and HePTP (50). Thus, activation of vascular MAP kinases by Ang II may be mediated, in part, through redox-dependent inactivation of PTPs.

Protein tyrosine kinases. Receptor- and non-receptor tyrosine kinases are also targets of ROS (40,41,47). Exogenous H_2O_2 induces tyrosine phosphorylation and activation of PDGFR and EGFR, probably due to ROS-mediated inhibition of dephosphorylation of PDGFR and EGFR by inactivation of membrane-associated PTPs. Oxygen intermediates, which are produced in response to tyrosine kinase receptor activation, are also involved in transactivation of PDGFR and EGFR by Ang II. This mechanism involves c-Src and Ras (32). In pathological conditions associated with oxidative stress, ROS may directly activate cell surface receptors, thereby amplifying the process of $\bullet O_2^-$ generation. Non-receptor tyrosine kinases such as Src, JAK2, STAT, p21Ras, Pyk2, and Akt, all of which are stimulated in response to Ang II and which have been implicated in cardiovascular remodeling and vascular damage, are regulated by ROS.

MAP kinases. MAP kinases are a family of ubiquitous proline-directed, protein-serine/threonine kinases, which participate in signal transduction classically associated with cell differentiation, cell growth and cell death (51). Of the major mammalian MAP kinases, ERK1/2, p38 MAP kinase and JNK are the best characterized. ERK1/2, phosphorylated by MEK1/2 (MAP/ERK kinase), is a key growth signaling kinase, whereas JNK and p38 MAP kinase, phosphorylated by MEK4/7 and MEK3/6, respectively, influence cell survival, apoptosis, differentiation, and inflammation. ERK5, regulated by MEK5, is involved in protein synthesis, cell cycle progression and cell growth. All MAP kinases are regulated, to varying degrees, by Ang II in vascular cells (2). Enhanced activation of vascular MAP kinases has been demonstrated in hypertension, atherosclerosis and diabetes and seems to be a major mechanism contributing to vascular damage associated with these conditions (51,52). MAP kinases are regulated by phosphorylation cascades and are strongly activated by ROS or by a mild oxidative shift of the intracellular thiol/disulfide redox state. Most studies have examined effects of exogenous H_2O_2 to activate MAP kinases (53). There are relatively few reports of endogenous ROS regulating the MAP kinase cascade. In VSMCs, intracellular ROS are critical for Ang II-induced activation of p38MAPK, JNK and ERK5, whereas phosphorylation of ERK1/2 appears to be redox-insensitive (54). However, serotonin-mediated ERK1/2 activation in smooth muscle cells is redox-sensitive, but in fibroblasts, it is not (40). Thus, redox-regulation of MAP kinases may be ligand- and cell-specific. Although MAP kinases are regulated by oxygen free radicals, they are probably not direct substrates of $\bullet O_2^-$ and H_2O_2.

Mechanisms whereby MAP kinases are activated by ROS are unclear, but MAP kinase phosphatases (MKP) are possible targets. Similar to PTPs, MKPs share a conserved essential redox-sensitive cysteine that confers catalytic activity. Inhibition of MKPs by ROS, through oxidative modification, results in activation of MAP kinases (41). In fact, decreased phosphatase activity has been linked to increased vascular ERK1/2 activation in hypertension (55). Other processes by which ROS influence MAP kinases may be through upstream activators, such as Src tyrosine kinases, the small GTPase Ras and PKC (17,18).

Calcium transport systems. In addition to influencing signaling pathways associated with cell growth and inflammation, ROS modulate intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), a major determinant of vascular contraction. Superoxide and H_2O_2 increase $[Ca^{2+}]_i$ in VSMCs and endothelial cells (56). These effects have been attributed to redox-dependent inositol-triphosphate-induced

Ca^{2+} mobilization, increased Ca^{2+} influx and decreased activation of Ca^{2+}-ATPase (57). Plasma membrane K^+ channels in VSMCs that control a hyperpolarization-elicited relaxation are opened by mechanisms associated with thiol oxidation by ROS (40). Recent studies reported that contractile responses to H_2O_2 are exaggerated in arteries from spontaneously hypertensive rats (SHR) compared with their normotensive counterparts (57). Findings from our laboratory demonstrated that H_2O_2-induced $[Ca^{2+}]_i$ transients are increased in VSMCs SHR (58). These data suggest that, in addition to impaired endothelium-dependent vasodilation (due to increased quenching of NO by $\bullet O_2^-$), redox-sensitive Ca^{2+} changes could contribute to altered vascular tone.

Processes whereby ROS influence signaling molecules

Two major processes have been identified whereby ROS influence signaling molecules: 1) oxidative modification of proteins and 2) changes in intracellular redox state (41).

Modification of proteins by oxidation. ROS can influence protein function and structure by various mechanisms: by altering important amino acid residues, by inducing protein dimerization and by interacting with metal complexes such as Fe-S moieties (41). Oxidative modification of amino acids within the functional domain of proteins occurs through many ways. The best characterized change involves cysteine residues. The sulfhydryl group (-SH) of a single cysteine residue may be oxidized to form sulfenic (-SOH), sulfinic ($-SO_2H$), sulfonic ($-SO_3H$), or S-glutathionylated (-SSG) derivatives. These changes alter the activity of the enzyme if the cysteine is within the catalytic domain or the ability of a transcription factor to bind DNA is located within its DNA binding motif (40,41). PTPs are directly inactivated by ROS-induced reversible oxidation of the catalytic site Cys^{215}. Other mechanisms by which ROS can influence proteins are by intramolecular disulfide bridge formation, where two or more cysteine residues within the same protein are oxidized, by protein dimerization through inter-molecular disulfide linkages, by diotyrosine formation and through metal-catalyzed oxidation by ROS.

Change in intracellular redox state. The intracellular compartment is generally maintained in a reduced state by the redox buffering capacity of intracellular thiols, particularly glutathione (GSH) and thioredoxin (TRX). These thiol redox systems counteract intracellular oxidative stress by reducing H_2O_2 and lipid peroxides. GSH peroxidases, which are selenoproteins, are located in the cytosol and mitochondria and use GSH to reduce H_2O_2 to produce GSSG: $H_2O_2 + 2GSH$ - glutathione peroxidase $\rightarrow\rightarrow 2H_2O + GSSG$.

As antioxidants, glutathione-dependent enzymes are particularly important because the intracellular concentrations are relatively high with glutathione in the millimolar range and thioredoxin in the micromolar range.

In addition to their antioxidant potential, GSH and TRX participate directly in redox signaling (59). GSH regulates signaling by modulating the levels of total GSH and the ratio of oxidized to reduced (GSH) forms. GSH can translocate to the nucleus where it regulates DNA binding of transcription factors (41). TRX is secreted by cells and was originally cloned as a cytokine-like factor. It is a low molecular weight (12 kDa) multifunctional protein with two redox-active cysteines within a conserved active site. TRX regulates activity of proteins by directly binding to them and by translocating to the nucleus to regulate gene expression through Ref1. Binding and activation of Ref1 by TRX induces DNA binding of the Jun-Fos complex to the AP-1 site to mediate transcription (41). NFκB and HIF-1 are also regulated by TRX. TRX has been implicated in apoptosis by inhibiting apoptosis signal regulating kinase (ASK1) (59). Although very little is known about the relationship between TRX

and Ang II, there is evidence that the TRX system is modulated by the renin angiotensin system, since ACE inhibition improves severity of myocarditis via redox regulation mechanisms involving TRX. In addition, in SHR, a model of Ang II-dependent hypertension, vascular TRX expression is impaired (60).

Reactive oxygen species as mediators of vascular damage

Under physiological conditions, vascular production of ROS and the consequent activation of redox-dependent signaling pathways and induction of redox-sensitive genes are tightly regulated. However, in pathological conditions, such as in hypertension, atherosclerosis, hyperlipidemia, hyperhomocysteinemia, and diabetes, where generation of ROS is increased and the renin angiotensin system may be upregulated, these redox-sensitive events may contribute to cellular processes involved in vascular dysfunction and structural remodeling (3-5).

Increased bioavailability of vascular ROS leads to VSMC growth, migration, collagen deposition, and altered MMP activity, important factors in arterial remodeling in cardiovascular disease (3-5) (Figure 5). In endothelial cells, oxidative excess induces apoptosis and aniokis (cell shedding), leading to endothelial cell loss and resultant impaired endothelial function. In addition, oxidative stress stimulates activation of transcription factors (e.g., NFκB and AP-1) and pro-inflammatory genes (cytokines, interleukins), upregulation of adhesion molecules (e.g., ICAM, VCAM, PECAM), stimulation of chemokine production (e.g., MCP-1) and recruitment of inflammatory cells (monocytes, macrophages), critical processes involved in vascular inflammation and injury (5,52). Increased vascular $\bullet O_2^-$ and H_2O_2 also impair endothelium-dependent relaxation, increase contractile reactivity and alter vascular tone. These effects may be mediated directly by elevating cytosolic Ca^{2+} concentration or indirectly by reducing concentrations of the vasodilator NO• (56).

Conclusions

Evidence is growing in support of ROS acting as signaling molecules in various cell types. The present review focuses on redox-

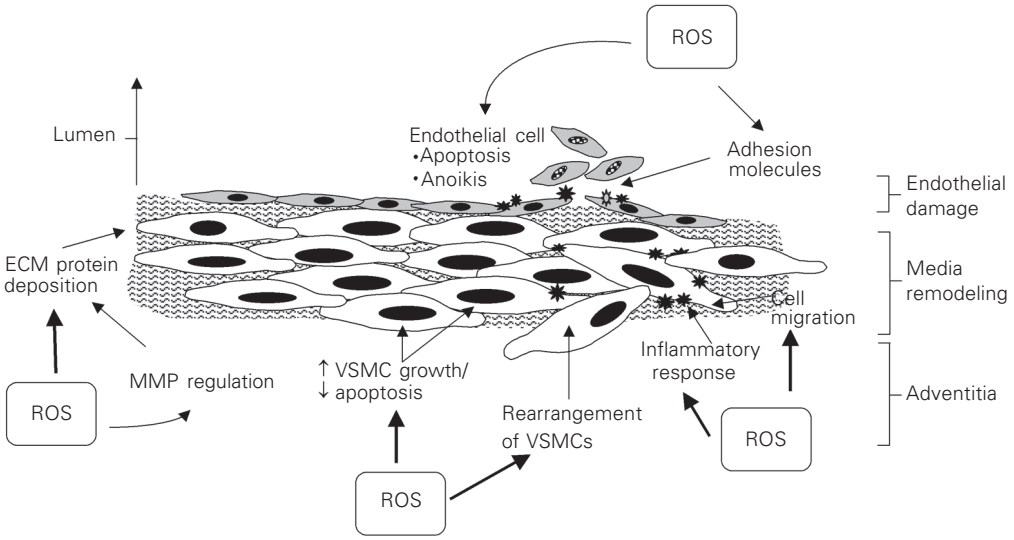

Figure 5. Vascular effects of reactive oxygen species (ROS). Increased bioavailability of ROS influences cellular processes leading to vascular smooth muscle cell (VSMC) growth, inflammation, migration and extracellular matrix (ECM) protein deposition as well as endothelial damage. MMP = matrix metalloproteinases.

sensitive pathways whereby Ang II mediates vascular changes associated with cardiovascular diseases. Although the processes underlying Ang II-generated ROS in the vasculature are becoming clearer, there is still a paucity of knowledge of how reactive oxygen intermediates function as second messengers in response to Ang II and how these redox-sensitive processes lead to vascular remodeling, endothelial dysfunction and inflammation. Future investigation in the field of redox signaling should elucidate how low levels of ROS act as signaling molecules in signal transduction cascades that regulate vascular function and maintain vascular integrity and what factors tip the balance so that high level oxidants act as damaging stress signals to induce vascular injury.

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