Modulation of Protein Kinase Activity and Gene Expression by Reactive Oxygen Species and Their Role in Vascular Physiology and Pathophysicsology

Kathy K. Griendling, Dan Sorescu, Bernard Lassègue, Masuko Ushio-Fukai

Abstract—Emerging evidence indicates that reactive oxygen species, especially superoxide and hydrogen peroxide, are important signaling molecules in cardiovascular cells. Their production is regulated by hormone-sensitive enzymes such as the vascular NAD(P)H oxidases, and their metabolism is coordinated by antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Both of these reactive oxygen species serve as second messengers to activate multiple intracellular proteins and enzymes, including the epidermal growth factor receptor, c-Src, p38 mitogen-activated protein kinase, Ras, and Akt/protein kinase B. Activation of these signaling cascades and redox-sensitive transcription factors leads to induction of many genes with important functional roles in the physiology and pathophysiology of vascular cells. Thus, reactive oxygen species participate in vascular smooth muscle cell growth and migration; modulation of endothelial function, including endothelium-dependent relaxation and expression of a proinflammatory phenotype; and modification of the extracellular matrix. All of these events play important roles in vascular diseases such as hypertension and atherosclerosis, suggesting that the sources of reactive oxygen species and the signaling pathways that they modify may represent important therapeutic targets. (Arterioscler Thromb Vasc Biol. 2000;20:2175-2183.)

Key Words: reactive oxygen species ■ vascular smooth muscle ■ endothelial cells ■ hypertension ■ atherosclerosis

Reactive oxygen species (ROS) are some of the newest additions to the family of second-messenger molecules. Although one ROS, nitric oxide (NO•), has been known for years to serve as a signaling molecule by activating guanylate cyclase, it has only recently become apparent that other ROS, including superoxide (O2•−) and hydrogen peroxide (H2O2), can alter the function of specific proteins and enzymes as well. In most cases, the mechanism by which these agents interact with their molecular targets is still unknown, but it is clear that they can mediate agonist-stimulated signaling. In this review, we will discuss redox-sensitive signaling cascades in vascular cells; their alteration by agonists, with particular attention to angiotensin II (Ang II); and their relevance to cardiovascular disease.

Production and Metabolism of ROS
Virtually all types of vascular cells produce O2•− and H2O2. In addition to mitochondrial sources of ROS, O2•− and/or H2O2 can be derived from xanthine oxidase, cyclooxygenase, lipoxygenase, NO synthase, heme oxygenases, peroxidases, hemoproteins such as heme and hematin, and NAD(P)H oxidases. Several investigators have shown that these latter enzymes, the membrane-associated NAD(P)H oxidase(s), are the primary physiological producers of ROS in vascular tissue. Of importance, the activity of these enzymes can be modulated by vasoactive hormones and the low-molecular-weight G protein rac-1, providing a critical characteristic of any second messenger: regulation of its production. Metabolism of ROS is also tightly controlled. Dismutation of O2•− by superoxide dismutase (SOD) produces the more stable ROS H2O2, which in turn is converted to water by catalase and glutathione peroxidase. Expression of antioxidant enzymes can be altered by hormones such as Ang II, tumor necrosis factor (TNF)-α, and interleukin (IL)-1β, thus profoundly affecting ROS levels. The tight regulation of both production and removal of ROS makes fluctuations in their levels transient, another requirement for second messengers. ROS may also act as an intracellular “rheostat,” closely modulating the activity of a discrete set of biochemical reactions. A schematic of the balance between oxidative and reductive states of the cell and the hormones, enzymes, and compounds that can alter this balance and thus, the overall response of the cell, is presented in Figure 1.

Vascular NAD(P)H Oxidases
The major sources of ROS in the vessel wall, the vascular NAD(P)H oxidases, are similar in structure to the neutrophil NADPH oxidase, which consists of 4 major subunits: a...
cytochrome b558, comprising gp91phox and p22phox, and 2 cytosolic components, p47phox and p67phox. A member of the low-molecular-weight G protein ras family participates in the assembly of the active complex. Table 1 summarizes the expression of the major phox subunits in vascular cells. Although the expression pattern of these molecules has been demonstrated, with the exception of p22phox in vascular smooth muscle cells (VSMCs) and endothelial cells and rac1 and p67phox in fibroblasts, it remains to be determined which subunits participate in functional complexes in specific cell types and/or whether as-yet-unidentified proteins take part in O2·− formation. If cardiovascular cells contain a neutrophil-like oxidase, it is essential to identify the electron transport moiety of the protein. Although gp91phox may serve this function in endothelial and adventitial cells, its apparent absence in SMCs suggests that a substitute must exist. Recently, several homologues of gp91phox have been cloned, and one of them, termed nox-1, for mitogenic oxidase (now known as nox-1, for NAPDH oxidase), has been shown to be expressed in VSMCs. In these cells, nox-1 mediates the proliferative response to serum, and nox-1 antisense attenuates O2·− production in response to platelet-derived growth factor (PDGF). Two other nox proteins have also been found: a 138-kDa protein (tox-1) that is the main, if not the sole, component of the thyroid oxidase, and a 578-aa amino acid protein, renox, that is expressed mainly in the kidney. Expression of these oxidases in vascular cells and their interaction with other phox subunits remain to be determined.

Regulation of ROS Production by Vasoactive Agonists and Mechanical Forces

There is good evidence for agonist-induced ROS production in both SMCs and endothelial cells. One of the first reports that the vascular NAD(P)H oxidase was hormone sensitive showed that Ang II treatment of SMCs increases intracellular O2·− production. Ang II–stimulated O2·− is converted to H2O2 as early as 1 minute after addition of hormone. Superoxide production in response to Ang II occurs when either NADH or NAPDH is used as a substrate and is inhibitable by diphenylene iodonium (DPI), a compound that binds to and inhibits flavin-containing oxidases; Tiron, an O2·− scavenger; N-acetylcysteine (NAC), which increases intracellular glutathione pools; and SOD. Treatment with antisense p22phox to depress NAPDH oxidase expression also blocks Ang II–induced O2·− production. Activation of this oxidase by Ang II appears to involve arachidonic acid metabolites, perhaps derived ultimately from phospholipase D–mediated phosphatidylcholine hydrolysis. Ang II also stimulates NAD(P)H-dependent O2·− production in endothelial cells and adventitial fibroblasts.

Other agonists and mechanical forces have also been shown to increase ROS production in vascular cells. PDGF, thrombin, TNF-α, and lactosylceramide activate NAD(P)H oxidase–dependent O2·− production in SMCs. Fibroblasts exhibit increased NADH- or NAPDH-driven O2·− production in response to TNF-α, IL-1, and platelet-activating factor. In endothelial cells, mechanical forces, including cyclic stretch and laminar and oscillatory shear stress, stimulate NAD(P)H oxidase activity. The upstream signals responsible for oxidase activation in each of these cell types with each of these stimuli remain to be established.

Signal Transduction Pathways Modulated by ROS

In order for ROS to modify the response of a cell to an agonist, it must affect specific signaling cascades. Over the past several years, many redox-sensitive proteins have been identified, and in some cases, it has been shown that hormonal activation is mediated by ROS. Often, both redox-sensitive and redox-insensitive pathways contribute to activation of a particular enzyme (Figure 2). The relationship between signaling cascades known to respond to ROS is depicted in Figure 2, and each pathway is discussed individually below.

Proximal Tyrosine Kinases

Growing evidence indicates that the epidermal growth factor receptor (EGF-R) and the PDGF receptor (PDGF-R) serve

TABLE 1. Expression (+) of Phagocytic Oxidase (phox) Components in Vascular Cells

<table>
<thead>
<tr>
<th></th>
<th>VSMCs</th>
<th></th>
<th>Endothelial Cells</th>
<th></th>
<th>Adventitial Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA</td>
<td>Protein</td>
<td>mRNA</td>
<td>Protein</td>
<td>mRNA</td>
<td>Protein</td>
</tr>
<tr>
<td>gp91phox</td>
<td>(13)</td>
<td>(13)</td>
<td>(13)</td>
<td>(13)</td>
<td>(13)</td>
</tr>
<tr>
<td>p22phox</td>
<td>(13, 112, 113)</td>
<td>(13)</td>
<td>(13)</td>
<td>(13)</td>
<td>(13)</td>
</tr>
<tr>
<td>p47phox</td>
<td>(13, 112, 113)</td>
<td>(13)</td>
<td>(13)</td>
<td>(13)</td>
<td>(13)</td>
</tr>
<tr>
<td>p67phox</td>
<td>(112, 113)</td>
<td>(112)</td>
<td>(112)</td>
<td>(114)</td>
<td>(114)</td>
</tr>
</tbody>
</table>

References are given in parentheses. ND indicates not determined.
although phosphorylation of the EGF-R by Ang II is redox sensitive, phosphorylation by EGF is not, suggesting that an even more proximal kinase than the EGF-R exists. Recently, we have shown that this kinase is c-Src.\(^3\text{4}\) c-Src is an important signaling molecule with many functions: it phosphorylates phospholipase C-\(\gamma\), forms complexes with the EGF-R,\(^3\text{2}\) paxillin,\(^3\text{8}\) and Janus kinase (JAK)-2.\(^3\text{9}\); and mediates activation of mitogen-activated protein kinases (MAPKs).\(^4\) In mouse fibroblasts, \(\text{H}_2\text{O}_2\) directly activates c-Src.\(^4\)\text{0}\) Moreover, Ang II–induced c-Src phosphorylation at both the autophosphorylation site (Y\(\text{418}\)) and the SH\(\text{\text{2}}\)-domain (Y\(\text{215}\)) is inhibited by antioxidants, suggesting that in VSMCs, \(\text{H}_2\text{O}_2\) is a proximal mediator of agonist-induced c-Src activation.\(^3\text{4}\)

Another signaling molecule that is activated quite early after receptor stimulation is the low-molecular-weight GTP-binding protein Ras. Ras has a dual role in redox-sensitive signaling: it mediates activation of the NADH/NADPH oxidase to generate intracellular ROS,\(^5\) and it is also activated by ROS in vivo and in vitro.\(^4\text{1}\text{–}\text{4}\text{3}\) ROS activate Ras via an oxidative modification of cysteine-118, leading to inhibition of the GDP-GTP exchange.\(^4\text{2}\) Moreover, ROS-triggered Ras activation induces recruitment of phosphatidylinositol 3'-kinase to Ras, an event that is required for activation of downstream signals such as Akt and MAPK (Figure 2 and below).\(^4\text{4}\)

Mitogen-Activated Protein Kinases

The MAPKs are a family of serine/threonine kinases that control cellular responses to growth, apoptosis, and stress signals. There are 4 main MAPKs, including extracellular signal–regulated kinases (ERK1/2), c-Jun N-terminal kinases (JNKs, also termed SAPKs), p38 MAPKs, and big MAPK-1. These proteins are the best studied in terms of their redox sensitivity. In SMCs, \(\text{H}_2\text{O}_2\) has been shown to activate p38 MAPK,\(^4\text{5}\text{, 4}\text{6}\) JNK,\(^4\text{6}\) and big MAPK-1.\(^4\text{7}\) Its effects on ERK1/2 are controversial, with some reports showing inhibition and others demonstrating stimulation.\(^4\text{5}\text{, 4}\text{6}\text{, 4}\text{8}\text{, 4}\text{9}\) In terms of agonist-induced activation of these enzymes, it has been clearly demonstrated that p38 MAPK and JNK activation by Ang II is inhibited by antioxidants (DPI, NAC), p22phox antisense, or overexpression of catalase.\(^4\text{5}\text{, 5}\text{0}\) Recently, it has been shown that arachidonic acid stimulates JNK via Rac-1–dependent \(\text{H}_2\text{O}_2\) production.\(^5\text{1}\) Because arachidonic acid is produced in response to many vasoactive hormones, this may represent a common mechanism of activation. Moreover, although PDGF-induced ERK1/2 phosphorylation is inhibited by incubation with catalase,\(^2\text{5}\) Ang II activation of these enzymes is not.\(^4\text{5}\text{, 4}\text{9}\text{, 5}\text{0}\)

In endothelial cells, \(\text{H}_2\text{O}_2\) activates p38 MAPK and its downstream target, MAPK-activated protein (MAPKAP) kinase 2/3, leading to phosphorylation of heat-shock protein 27 (Hsp27).\(^5\text{2}\text{, 5}\text{3}\) ERK1/2 activation also seems to be redox sensitive in this cell type, based on the observation that shear stress–induced ERK1/2 phosphorylation is inhibited by antioxidants and dominant-negative Rac-1.\(^4\text{4}\) In neonatal rat ventricular myocytes, all 3 MAPKs (ERK1/2, p38 MAPK, and JNK) have been demonstrated to be activated by \(\text{H}_2\text{O}_2\).\(^5\text{5}\) Thus, regulation of MAPK activity by ROS varies not only among family members but also among cells.
Akt
The recently identified serine/threonine kinase Akt/protein kinase B has been shown to play a key role in many cellular processes, including cell survival and protein synthesis. Akt inhibits glycogen synthase kinase 3 and activates p70S6K and the transcription factors activating protein (AP)-1 and E2F. Similar to p38 MAPK, both exogenous H2O2 and Ang II activate Akt in SMCs. Ang II–induced Akt phosphorylation is inhibited by DPI or overexpression of catalase, suggesting a role for NAD(P)H oxidase–derived ROS in agonist-induced Akt activation. H2O2 stimulation of Akt has also been reported in other nonvascular cell types, including NIH3T3 fibroblasts, human embryonic kidney 293 cells, and HeLa and Jurkat cells. It is noteworthy that Konishi et al demonstrated that H2O2-induced Akt activation caused association with Hsp27, which itself is also phosphorylated by H2O2. Furthermore, MAPKAP kinase-2, a substrate of p38 MAPK, can phosphorylate Akt in vitro, raising the possibility that H2O2 may phosphorylate both Akt and Hsp27 by activation of p38 MAPK.

Other Candidate Redox-Sensitive Enzymes
Most likely, we have only scratched the surface of the cadre of oxidant-sensitive signaling pathways. Many proteins, including phospholipase D, Fyn, proline-rich tyrosine kinase (Pyk) 2, JAK2, and signal transducer and activator of transcription (STAT) 1, appear to be redox sensitive, based on their activation by addition of exogenous ROS. For example, H2O2 and lipid hydroperoxides activate phospholipase D in endothelial cells. In mouse fibroblasts, H2O2 activates JAK2 via Fyn kinase, resulting in the stimulation of Ras activity. Pyk2 has also been reported to be redox sensitive, because H2O2 and the strong oxidant diamide both increase Pyk2 phosphorylation. Furthermore, PDGF-induced STAT activation is inhibited by antioxidants such as NAC and DPI. Although, for the most part, the role of ROS in activation of these pathways by agonists has not been studied, their clear relationship with ROS suggests that they are potentially among the proteins that mediate redox-sensitive physiological responses.

Regulation of Gene Expression by ROS
Because multiple hormones and growth factors alter tissue and intracellular levels of ROS and various critical signaling pathways are activated by ROS, it is not surprising that many cardiovascular-related genes are redox sensitive. Perusal of Table 2 indicates that ROS regulate several general classes of genes, including adhesion molecules and chemotactic factors, antioxidant enzymes, and vasoactive substances. Some of these are clearly an adaptive response, such as the induction of SOD and catalase by H2O2. Most redox-sensitive genes have been identified because they are responsive to externally applied oxidant stress; only a few have been demonstrated to be downstream of an endogenous source of ROS, such as the NAD(P)H oxidase. These include TNF-α and lactosylceramide induction of intercellular adhesion molecule (ICAM)-1 and Ang II, PDGF, and TNF-α stimulation of monocyte chemotactic protein (MCP)-1. In contrast, stimulation of MCP-1 by IL-1β in VSMCs is not affected by antioxidants, suggesting that the control of gene expression by ROS is both stimulus and tissue specific.

Induction of several genes by cytokines is inhibited by NO donors, including vascular cell adhesion molecule (VCAM)-1, ICAM-1, and monocyte colony-stimulating factor (M-CSF). This is an interesting mechanism of regulation because NO appears to act in a cGMP-independent manner to inhibit expression at the level of transcription. Not only can NO alter the activity and expression of transcription factors, but also it scavenges O2•− to form peroxynitrite, thus modulating O2•−-dependent transcription as well.

Regulation of gene expression by oxidant stress occurs at various levels. In some cases, regulation of the gene is redox sensitive owing to the susceptibility of upstream signaling pathways to ROS. For example, induction of early growth response (Egr)-1 by cyclic strain has been shown to depend on redox-sensitive activation of the Ras-Raf-ERK1/2 pathway. Moreover, H2O2-induced AP-1 binding in porcine aortic endothelial cells requires activation of Src. In other cases, ROS mediate increased turnover, expression, or transcription of specific transcription factors, thus modifying their activity. This mechanism has been shown to be effective for both the nuclear factor (NF)-κB and AP-1 transcription factors. Hydroperoxy fatty acids and H2O2 increase the expression of Fos and Jun, 2 proteins that form heterodimers and activate AP-1. The inhibitory factor that binds NF-κB and causes retention of this transcription factor in a cytoplasmic, inactive form. The turnover of IκB protein is also oxidant sensitive: antioxidants can prevent agonist-stimulated IκB phosphorylation and degradation. Conversely, H2O2 increases nuclear translocation of NF-κB, contributing to the induction of genes responsive to this transcription factor.

An additional level of redox regulation of gene expression is that the affinity of certain transcription factors for their cognate DNA-binding sites can be directly modified by ROS. This mechanism was first identified in bacteria, where excess H2O2 interacts with the oxyR regulon, and O2•− or NO activates the soxRS regulon to control the expression of a subset of genes, including MnSOD and aconitate. The oxyR-binding motif has also been shown to function as a redox-sensitive transcriptional enhancer in mammalian cells. Since then, several mammalian transcription factors have been shown to be directly modified by ROS or by reducing proteins that modify cysteine residues involved in DNA binding. Transcription factors in this category include AP-1, NF-κB, and most likely hypoxia-inducible factor (HIF)-1. Both Fos and Jun have a conserved cysteine in a basic motif that, when oxidized, interferes with the binding of these proteins to AP-1 consensus sequences. Conversely, if Fos/Jun heterodimers are bound to AP-1, they cannot be oxidized. The oxidation state of these important proteins is controlled by redox factor (REF)-1, a protein that, in cooperation with thioredoxin, promotes the cycling of the critical cysteines between reduced and oxidized forms. Thioredoxin also regulates HIF-1–dependent transcription and modifies the DNA binding and transcriptional activity of NF-κB by reducing cysteine 62. These studies clearly
indicate the importance of the nuclear redox state in regulating gene expression.

**Role of ROS in Vascular Physiology and Pathophysiology**

The intracellular and extracellular production of ROS and the consequent activation of specific signaling pathways and induction of redox-sensitive genes coordinate several integrated physiological responses in cardiovascular tissue, including growth of smooth muscle, induction of an inflammatory response, impairment of endothelium-dependent relaxation, and cardiac hypertrophy. Each of these responses, when uncontrolled, contributes to vascular disease.

**Vascular Smooth Muscle Growth, Hypertrophy, and Apoptosis**

A characteristic of hypertension is hypertrophy of large vessels. We have demonstrated that Ang II–induced hypertrophy of SMCs is dependent on intracellularly produced H₂O₂, which is derived, at least in part, from an NAD(P)H oxidase. Ang II–induced hypertrophy can be inhibited by DPI attenuation of NAD(P)H oxidase activity by transfection of antisense p22phox, and catalase overexpression.

Similar findings were reported for cardiac myocytes, in which Ang II–induced hypertrophy was associated with intracellular production of ROS and was blocked by antioxidants.

Other vascular disorders such as restenosis have a significant proliferative component, resulting from SMC and/or fibroblast migration and multiplication in the neointima.

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**TABLE 2. Redox Sensitivity of Gene Expression in Cardiovascular Cells**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cell Type</th>
<th>Stimulus</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCAM-1</td>
<td>Endothelial cells</td>
<td>TNF-α, IL-1α, IL-1β, IL-4</td>
<td>116, 117</td>
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<tr>
<td>ICAM-1</td>
<td>Endothelial cells</td>
<td>TNF-α, NO, lactosylceramide</td>
<td>26, 71, 73, 116</td>
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<tr>
<td>E-selectin</td>
<td>Endothelial cells</td>
<td>IL-1α, LPS, PMA, TNF-α</td>
<td>73, 117, 118</td>
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<tr>
<td>MCP-1</td>
<td>Mesangial cells</td>
<td>TNF-α</td>
<td>24, 72, 106, 119</td>
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<tr>
<td></td>
<td>VSMCs</td>
<td>PDGF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VSMCs</td>
<td>Ang II</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VSMCs</td>
<td>TNF-α</td>
<td></td>
</tr>
<tr>
<td>MCSF</td>
<td>Endothelial cells</td>
<td>TNF-α, ox-LDL</td>
<td>75, 76, 119</td>
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<td>eNOS</td>
<td>Endothelial cells</td>
<td>Xanthine/xanthine oxidase</td>
<td>120</td>
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<tr>
<td>iNOS</td>
<td>Mesangial cells</td>
<td>IL-1β</td>
<td>121</td>
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<tr>
<td>Cu/Zn-SOD</td>
<td>Endothelial cells</td>
<td>H₂O₂</td>
<td>70</td>
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<tr>
<td>Catalase</td>
<td>Endothelial cells</td>
<td>H₂O₂</td>
<td>70</td>
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<td>Glutathione peroxidase</td>
<td>Endothelial cells</td>
<td>H₂O₂</td>
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<td>Mn-SOD</td>
<td>Endothelial cells</td>
<td>Thioredoxin</td>
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<td>HO-1</td>
<td>Endothelial cells</td>
<td>H₂O₂, shear stress</td>
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<td>Macrophages</td>
<td>OX-LDL</td>
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<td></td>
<td>VSMCs</td>
<td>PDTC</td>
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<td>COX-2</td>
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<td></td>
<td>VSMCs</td>
<td>Catalase overexpression</td>
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<tr>
<td>HSP-70</td>
<td>Endothelial cells</td>
<td>H₂O₂</td>
<td>70, 125</td>
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<td></td>
<td>Xanthine/xanthine oxidase</td>
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<tr>
<td>Scavenger receptor</td>
<td>VSMCs</td>
<td>PMA, H₂O₂/Oxidized</td>
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<tr>
<td></td>
<td>Macrophages</td>
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<td>IL-8</td>
<td>Microvascular endothelial cells</td>
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<td>127</td>
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<tr>
<td>HB-EGF</td>
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<td>H₂O₂</td>
<td>128, 129</td>
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<td></td>
<td>VSMCs</td>
<td>Methylglyoxal</td>
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<tr>
<td>Atrial natriuretic factor</td>
<td>Cardiac myocytes</td>
<td>Ouabain</td>
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<td>VEGF</td>
<td>Endothelial cells</td>
<td>H₂O₂</td>
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<tr>
<td></td>
<td>VSMCs</td>
<td>H₂O₂, 4-hydroxynonenal</td>
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e/i NOS indicates endothelial/inducible nitric oxide synthase; HO-1, heme oxygenase-1; COX-2, cyclooxygenase-2; HB, heparin binding; LPS, lipopolysaccharide; PMA, phorbol myristate acetate; ox, oxidized; and PDTC, pyrrolidine dithiocarbamate.
Sundaresan et al. demonstrated a clear requirement for H2O2 in PDGF-induced proliferation. Migration in response to this agonist is also inhibited by catalase, suggesting that it, too, is mediated by ROS. Similar results were found by Brown et al., who showed that overexpression of catalase in SMCs not only inhibited serum-induced [3H]thymidine incorporation and proliferation but also promoted apoptosis. Phenylephrine-induced proliferation of rabbit aortic SMCs has also been shown to require H2O2. Proof that balloon angioplasty increases oxidant stress has been provided in 2 studies. Within 30 minutes after injury, glutathione levels fall by 63%, coincident with medial smooth muscle apoptosis, suggesting that this early step in the response to injury is associated with severe oxidant stress. Importantly, administration of NAC or pyrrolidine dithiocarbamate prevents the glutathione loss and the smooth muscle apoptosis. In another study, Nunes et al. showed that vascular O2•− was increased 2.5-fold in injured arteries compared with uninjured controls. Moreover, treatment with either probucol or the combination of vitamins C and E normalized O2•− levels and partially suppressed neointimal formation. Davies et al. have recently reported that p38 MAPK is upregulated after injury, suggesting that this signaling pathway might also be a redox-sensitive target in vivo.

Endothelial Dysfunction

Endothelial dysfunction is a hallmark of multiple vascular diseases, including hypertension, atherosclerosis, and diabetes mellitus. Impaired endothelial function has several consequences, the most important of which is decreased endothelium-dependent vasodilation. The endothelial cell redox rheostat is primarily regulated by the dynamic production of and interaction between NO− and O2•−. NO− is the most potent endogenous vasodilator and inhibits smooth muscle proliferation and migration, adhesion of leukocytes to the endothelium, and platelet aggregation. In cholesterol-fed rabbits, O2•− is increased in the aorta, and treatment with polyethylene glycol–SOD reverses the impairment in endothelial-dependent relaxation. In the same animal model, treatment with probucol (a lipid-lowering agent with potent antioxidant properties) corrects endothelial dysfunction and lowers O2•−. Impaired endothelium-dependent vasodilation also occurs in hypertension, such as that produced by infusion of angiotensin II, restriction of blood flow to 1 kidney, and treatment of deoxycorticosterone acetate-salt. The endothelial dysfunction that accompanies Ang II infusion or deoxycorticosterone acetate-salt can be corrected by administration of liposomal or matrix-targeted SOD, providing further proof that ROS, and specifically O2•−, are involved in this response.

The Inflammatory Response

Another consequence of endothelial dysfunction and SMC activation is increased monocyte adhesion, foam cell formation, and thrombosis. As noted above, pro-oxidant agonists such as Ang II and TNF-α induce the expression of proinflammatory molecules such as VCAM-1, MCP-1, and the thrombin receptor. Each of these molecules is in turn redox sensitive, and in the case of MCP-1 and the thrombin receptor, a role for ROS in Ang II–mediated gene expression has been demonstrated.

Matrix Remodeling

Collagen degradation depends on the activity of enzymes known as metalloproteinases (MMPs). MMP-2 (gelatinase A, which degrades collagen IV from the basal membrane) and MMP-9 (gelatinase B, which acts on collagen I fibers) are secreted by macrophages and vascular myocytes in an inactive form. MMP-9 expression is increased in the shoulder region of atherosclerotic plaques; ie, in the sites prone to plaque rupture. Rajagopalan et al. demonstrated that pro–MMP-9 and pro–MMP2 secreted into the medium of cultured human SMCs are activated by ROS. Moreover, NAC treatment prevents MMP-9 expression and activation in hypercholesterolemic rabbits, suggesting a mechanism for how antioxidants may contribute to plaque stabilization.

Conclusions and Future Directions

Much remains to be learned concerning the signaling pathways and genes that are regulated by ROS. Because redox-sensitive responses appear at times to be cell specific, it will be important to identify the sources of oxidant stress in each cell, the mechanism of regulation of antioxidant enzymes, and the effect of ROS on signaling pathways specific to the function of that particular cell and to gain further insight into the physiological responses affected by oxidant stress. An understanding of these mechanisms will enable us to devise therapeutic strategies to target specific cellular events contributing to vascular disease.

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References


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