NAD(P)H Oxidase : Role in Cardiovascular Biology and Disease

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Circ Res. 2000;86:494-501
doi: 10.1161/01.RES.86.5.494
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571

MiniReviews

This MiniReview is part of a thematic series on **Oxidant Signaling in Cardiovascular Cells**, which includes the following articles:

NAD(P)H Oxidase: Role in Cardiovascular Biology and Disease

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Oxygen Radicals and Endothelial Dysfunction

Oxidant Signaling in Vascular Cell Growth, Death, and Function

Antioxidant Vitamins and Clinical Studies

Vascular Oxygen Species Generation

David G. Harrison, Guest Editor

NAD(P)H Oxidase
Role in Cardiovascular Biology and Disease

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

Abstract—Reactive oxygen species have emerged as important molecules in cardiovascular function. Recent work has shown that NAD(P)H oxidases are major sources of superoxide in vascular cells and myocytes. The biochemical characterization, activation paradigms, structure, and function of this enzyme are now partly understood. Vascular NAD(P)H oxidases share some, but not all, characteristics of the neutrophil enzyme. In response to growth factors and cytokines, they produce superoxide, which is metabolized to hydrogen peroxide, and both of these reactive oxygen species serve as second messengers to activate multiple intracellular signaling pathways. The vascular NAD(P)H oxidases have been found to be essential in the physiological response of vascular cells, including growth, migration, and modification of the extracellular matrix. They have also been linked to hypertension and to pathological states associated with uncontrolled growth and inflammation, such as atherosclerosis. **(*Circ Res*. 2000;86:494-501.)**

Key Words: NAD(P)H oxidase ■ reactive oxygen species ■ superoxide ■ vascular cells

Recent work has provided a broad experimental base supporting a critical role for oxidative stress in the normal functioning of cardiac and vascular cells, as well as in the pathogenesis of vascular disease. The concept of oxidation of LDL as an initiating event in vascular lesion formation has been extended to encompass numerous other roles for oxidation-reduction reactions in cardiovascular biology. An emerging common theme of these studies is that an NAD(P)H oxidase is a major source of superoxide in vascular cells and myocytes. A plethora of experimental data has recently been generated concerning the mechanisms of activation of this enzyme, its role in normal cell functioning, and its participation in cardiovascular disease.

NAD(P)H Oxidase

It has been known for years that vascular and cardiac tissues are rich sources of reactive oxygen species (ROS), including superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and nitric oxide ($NO^{\cdot}$). Virtually every cell type in the vascular wall has been shown to produce and be regulated by ROS (for review see References 1 and 2). Early work identified xanthine oxidase as a prime source of endothelium-dependent $O_2^{\cdot -}$ production.[3,4] It has subsequently been established that smooth muscle cells and fibroblasts account for the majority of $O_2^{\cdot -}$ produced in the normal vessel wall.[5–8] Currently, attention is focused on NAD(P)H oxidases as critical determinants of the redox state of blood vessels and the myocardium.

Biochemical Characteristics

The NAD(P)H oxidases of the cardiovascular system are membrane-associated enzymes that catalyze the 1-electron reduction of oxygen using NADH or NADPH as the electron donor.

$$NAD(P)H + 2O_2 \rightarrow NAD(P)^+ + H^+ + 2O_2^{\cdot -}$$

Received September 2, 1999; accepted January 5, 2000.

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This enzymatic activity has been shown to compose the major oxidase in vascular tissue[8–10] and in cardiac cells,[11] as compared with production of ROS from xanthine oxidase, arachidonic acid, and mitochondrial oxidases. In most studies, NADH is proposed to be the preferred substrate,[6,8,12–14] but some investigators find NADPH-driven $O_2^{\cdot-}$ generation to predominate.[5,10] In the only study to approximate K_m values for the vascular smooth muscle cell (VSMC) enzyme, De Keulenaer et al[15] found that the K_m for NADH was ≈10 μmol/L. Recent work suggests that this substrate preference may depend somewhat on the method of detection. Superoxide production in nonphagocytic cells is often measured using lucigenin, an acridylium dinitrite compound that emits light on reduction and interaction with $O_2^{\cdot-}$.[16] Lucigenin has been criticized because of its ability to undergo redox cycling, in particular when NADH is used as a substrate.[17] Low levels of lucigenin (<10 μmol/L) do not suffer from this drawback,[17] and under these conditions, NADPH appears to be the major substrate of the vascular enzymes (D. Sorescu, K.K. Griendling, unpublished observations, 1999). Analysis with electron spin resonance spectroscopy using the spin trap 5-diethoxyphosphoryl-5-methyl-pyrroline-*N*-oxide (DEPMPO) indicates that NADPH and NADH are equally good substrates in VSMCs (D. Sorescu, K.K. Griendling, unpublished observations, 1999) but that NADH-driven $O_2^{\cdot-}$ production predominates in endothelial cells (ECs) (D. Harrison, personal communication, June 1999). Further investigation into the substrates utilized by these enzymes under different physiological conditions is imperative.

Vascular NAD(P)H oxidase activity has been better studied than the activity of the cardiac enzyme. These enzymes appear to reside in cell membranes. We and others have observed NADH- or NADPH-driven $O_2^{\cdot-}$ production in the 100 000-g membrane fraction, suggesting a plasma membrane or microsomal localization.[8,9,18,19] There is some controversy regarding the orientation of these enzymes. By analogy with the neutrophil enzyme (see below), the oxidase should span the membrane, utilizing intracellular NADH or NADPH and transferring electrons across the membrane to extracellular oxygen. In support of this concept, $O_2^{\cdot-}$ release has been observed from ECs and fibroblasts.[18,20] However, in VSMCs, $O_2^{\cdot-}$ and H_2O_2 production appears to be mainly intracellular,[6,21] and in fibroblasts, an NADH-driven ectoenzyme has been identified.[22] It has also been shown that addition of NADPH or NADH extracellularly augments $O_2^{\cdot-}$ formation,[23] calling into question whether nonphagocytic cells exhibit the classical inside-out orientation of neutrophils.

The cardiovascular NAD(P)H oxidases are low-output, slow-release enzymes of which the biochemical characteristics differ considerably from those of the neutrophil NADPH oxidase. Estimates of $O_2^{\cdot-}$ production in vascular cells suggest that the capacity of these enzymes is about one third that of the neutrophil.[24] Furthermore, the vascular enzymes appear to have a moderate constitutive activity that is absent in phagocytes.[7] The kinetics of activation on cellular stimulation are also unique; $O_2^{\cdot-}$ is produced in minutes to hours in ECs, VSMCs, and fibroblasts,[6,25,26] in contrast to the almost instantaneous release seen in neutrophils. Despite these differences, the neutrophil and vascular enzymes share some characteristics; both are inhibited by diphenylene iodonium (DPI), an inhibitor of flavin-containing oxidases, and both are stimulated by agonists and arachidonic acid.[24]

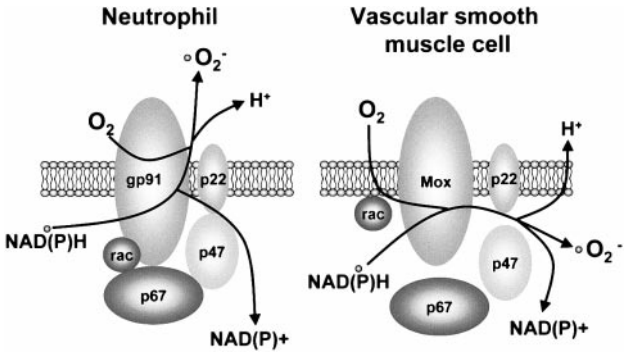

Figure 1. Structure of the NAD(P)H oxidase. Left, Functional structure of the neutrophil NAD(P)H oxidase. gp91phox and p22phox form the electron transfer component of the oxidase, and p47phox and p67phox are cytosolic components that interact with these 2 proteins to modulate its activity. The low molecular weight G protein rac also serves a regulatory function. Right, Components of the neutrophil oxidase that have been identified in VSMCs. The functional interaction among these subunits remains to be determined.

Structure

The above comparison suggests that although the cardiovascular oxidases are similar to the neutrophil NADPH oxidase, they represent a unique family of enzymes (Figure 1). The neutrophil oxidase consists of 4 major subunits: a plasma membrane spanning cytochrome b558 composed of a large subunit gp91phox and a smaller, p22phox subunit and 2 cytosolic components p47phox and p67phox. The low molecular weight G protein rac2 (in some cells rac1) participates in assembly of the active complex, and a second G protein, rap1A, copurifies with the enzyme and may participate in deactivation.[27] Much effort has been directed at identifying which of these subunits are present in cardiovascular cells and in determining whether a similar multimeric complex might be responsible for oxidase activity (Table). Using molecular biological approaches, mRNAs for gp91phox, p22phox, p47phox, and p67phox have been demonstrated in ECs[14,28] and adventitial cells.[7] VSMCs and mesangial cells appear to express p22phox, p47phox, and rac1, but not gp91phox,[19,29,30] with the possible exception of pulmonary artery smooth muscle, in which gp91phox has been detected.[31] p67phox has been found in mesangial cells[29] but has been reported to be absent in VSMCs.[19] The phox content of fibroblasts is controversial; in some cases, all subunits have been detected,[7] whereas in others, only p22phox, p47phox, and p67phox were identified.[32,33]

Proving a role for each of these components in oxidase activity has been challenging. Several years ago, we showed that antisense p22phox stably transfected into cultured VSMCs resulted in a 50% decrease in angiotensin

Evidence for the Presence of Oxidase Subunits in Isolated Nonphagocytic Cells

	gp91phox		mox		p22phox		rac		p47phox		p67phox		
	RNA	Protein	RNA	Protein	RNA	Protein	RNA	Protein	RNA	Protein	RNA	Protein	References
Neutrophil	+++	+++	−	ND	+++	+++	+++	+++	+++	+++	+++	+++	27, 36
VSMC	−/+	−	++	ND	+++	+++	+++	+++	+	+?	+	?	19, 30, 31, 36
EC	+	−/+	ND	ND	+	+	+	+	−/+	−/+	+	−/+	14, 28
Fibroblast	−/+	−/+	−	ND+	+	+	+	+	+	+	+	+	7, 32–34, 36
Mesangial cell	−	−?	ND	ND	+	+	+	+	+	+	+	+	29, 93

ND indicates not determined; −, not expressed; +, expressed; and +++, strongly expressed.

II–stimulated NAD(P)H oxidase activity.[30] Patterson et al[19] reported a good correlation between thrombin-induced $O_2^{\cdot-}$ production and translocation and expression of p47phox and rac2, providing indirect evidence that these 2 subunits participate in the active enzymatic complex. Using negative dominant rac1, Irani et al[34] demonstrated that $O_2^{\cdot-}$ production in fibroblasts was dependent on functional rac1. Similarly, Pagano et al[7] recently suggested that p67phox was required for fibroblast NAD(P)H oxidase activity by showing that immunodepletion of p67phox resulted in a decrease in $O_2^{\cdot-}$ production. In ECs, phox components are poorly detected at the protein level, and cytochrome b558 is not measurable spectrophotometrically,[14] so that functional evidence in this cell type is lacking. The ability of DPI to inhibit EC $O_2^{\cdot-}$ production, however, suggests that a similar enzyme complex may be responsible.

Despite these advances, it remains to be determined in each of these cells which subunits form functional complexes and whether as-yet-unidentified proteins participate in $O_2^{\cdot-}$ formation. If cardiovascular cells contain a neutrophil-like functional oxidase, it is essential to demonstrate the existence of the electron transport moiety of the protein. In neutrophils, it has been suggested that the 2 components of cytochrome b558, gp91phox and p22phox, stabilize each other.[35] The finding that some nonphagocytic cells express p22phox in the absence of gp91phox raises the possibility that there are gp91phox isoforms that serve a similar function in these cells. One of the first indications that this might be the case came from a seminal observation by Meier et al[33] that $O_2^{\cdot-}$ production was normal in fibroblasts from a chronic granulomatous disease patient whose neutrophil oxidase functioned at only 10% normal capacity. They further showed that the fibroblast cytochrome was immunologically distinct from that of phagocytes. Recently, a homologue of gp91phox (termed mox-1, for mitogenic oxidase) was cloned from human colon carcinoma and rat aortic smooth muscle cells.[36] Human mox1 has 56% identity with human gp91phox, and importantly, all of the major functional domains (pyridine binding sites, flavin nucleotide binding sites, and potential heme binding sites) are completely conserved. Furthermore, the hydrophobic profiles of mox1 and gp91phox are nearly identical, suggesting similar cellular localization. Overexpression of mox1 in fibroblasts leads to an increase in $O_2^{\cdot-}$ production and a transformation in phenotype to increased, anchorage-independent cell growth. Moreover, expression of mox1 antisense in VSMCs results in a decrease in serum-stimulated NADPH-driven $O_2^{\cdot-}$ production and a decrease in cell growth. Fragments of other gp91phox homologues have been identified,[36] but their functional role remains to be established. The discovery of mox1 indicates that it is likely that the vascular NAD(P)H oxidases belong to a novel family of growth factor–responsive oxidative enzymes.

Mechanism of Activation

One of the most important attributes of the cardiovascular oxidase is its responsiveness to hormones, hemodynamic forces, and local metabolic changes. It has been clearly demonstrated that the activity of the vascular oxidase is increased by the important vasoactive agonist angiotensin II. Angiotensin II increases NADH- and NADPH-driven $O_2^{\cdot-}$ production in cultured VSMCs[6] and aortic adventitial fibroblasts[25] and increases DPI-inhibitable ROS production in mesangial cells.[37] Thrombin, platelet-derived growth factor (PDGF), tumor necrosis factor-α (TNF-α), and lactosylceramide also stimulate NAD(P)H oxidase-dependent $O_2^{\cdot-}$ in VSMCs.[15,19,38–40] With regard to metabolic changes, reoxygenation and the accompanying increase in lactate stimulate NADH oxidase activity in cardiac myocytes.[11] Fibroblasts exhibit increased NADH- or NADPH-dependent $O_2^{\cdot-}$ production in response to TNF-α, interleukin-1, and platelet-activating factor.[41,42] Moreover, transforming growth factor-β1 causes a slow, sustained H_2O_2 release that is inhibitable by DPI, but it has no effect on $O_2^{\cdot-}$ production, raising the possibility that the NAD(P)H oxidase might directly participate in the 2-electron reduction of oxygen to H_2O_2.[43] In ECs, mechanical forces stimulate NAD(P)H oxidase activity,[44,45] but agonist responsiveness remains to be established. Both unidirectional and oscillatory shear stresses increase $O_2^{\cdot-}$ production. Exposure of cultured human umbilical vein ECs to 5 or 20 dyne/cm^2 laminar shear stress results in a transient elevation in $O_2^{\cdot-}$ formation[45,46] that is apparently derived from activation of an NAD(P)H oxidase, whereas oscillatory shear causes a sustained (up to 24 hours) increase in oxidase activity.[45]

Regulation of oxidase activity in cardiovascular cells occurs at at least 2 levels. First, activation of the oxidase can be mediated by intracellular second messengers, including calcium.[42] Although neutrophil oxidase activity is regulated by protein kinase C,[47] it is unclear whether this

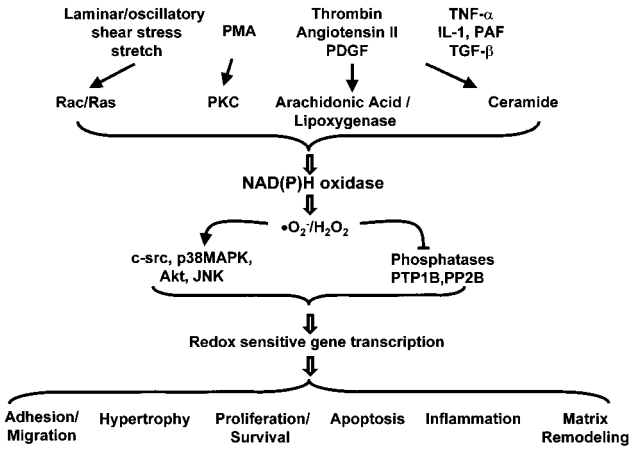

Figure 2. Model of oxidase activation and functional consequences in cardiovascular cells. Multiple vasoactive agonists and hemodynamic forces activate the NAD(P)H oxidase via signaling pathways that include rac/ras, arachidonic acid metabolites, and ceramide. Production of $O_2^{\cdot-}$ and its metabolite H_2O_2 lead to activation of redox-sensitive kinases and potentially inactivation of specific phosphatases to modulate gene expression. The overall biological impact of NAD(P)H oxidase activation thus involves adhesion and migration of monocytes/macrophages, hypertrophy of VSMCs, proliferation and survival of several types of vascular cells, apoptosis, inflammation, and remodeling of the extracellular matrix. PMA indicates phorbol 12-myristate 13-acetate; PKC, protein kinase C; IL-1, interleukin-1; PAF, platelet-activating factor; and TGF-β, transforming growth factor-β.

kinase participates in activation of nonphagocytic oxidases. Phorbol 12-myristate 13-acetate, a protein kinase C activator, has no effect on adventitial $O_2^{\cdot-}$ production,[7] but it increases NAD(P)H oxidase activity in VSMCs (D. Sorescu, K.K. Griendling, unpublished observations, 1999). It has recently been shown that lipoxygenase metabolites of arachidonic acid mediate angiotensin II stimulation of the NAD(P)H oxidase in VSMCs,[48] but the upstream signaling pathways remain to be defined. Secondly, oxidase activity can also be modulated by upregulation of the component mRNAs. For example, TNF-α increases NAD(P)H oxidase activity in VSMCs over 24 hours, an event that is dependent on increased transcription of p22phox.[15] p22phox mRNA and $O_2^{\cdot-}$ production are upregulated in the aortas of rats made hypertensive by angiotensin II infusion,[49] and angiotensin II increases the expression of p67phox in adventitial fibroblasts.[25] Conversely, Marumo et al[50] showed that dexamethasone inhibited PDGF-stimulated $O_2^{\cdot-}$ and H_2O_2 production and decreased p22phox mRNA expression in VSMCs but not in neutrophils.

Biological Effects of NAD(P)H Oxidase Activation

ROS and NAD(P)H oxidases have been implicated in numerous cellular processes and vascular diseases (Figure 2). These enzymes have been proposed to be oxygen sensors and to participate in hypoxic vasoconstriction[12] and/or vasodilation,[51] but this has recently been called into question by Archer et al,[31] who showed that oxygen sensing was normal in gp91phox-deficient mice. The discovery of gp91phox homologues may help to settle this controversy. Production of $O_2^{\cdot-}$ in the vessel wall has been shown to inactivate nitric oxide, leading to the production of peroxynitrite and impaired endothelium-dependent vasodilation[52,53]; oxidize LDL[54]; increase adhesion molecule expression in ECs resulting in monocyte infiltration[55]; and activate matrix metalloproteinases, leading to vascular remodeling.[56] NAD(P)H oxidase-derived $O_2^{\cdot-}$ and H_2O_2 are intimately involved in the growth response of VSMCs and fibroblasts[6,21,30,34,39] and also participate in VSMC migration[39] and, in some cases, cellular apoptosis.[57] These ROS are thus functioning as signaling molecules to mediate specific cellular responses.

NAD(P)H-Derived ROS as Second Messengers

Stimulation of cell surface receptors with growth factors or agonists activates various cellular signaling pathways, including protein tyrosine kinases, serine/threonine kinases, phospholipases, and Ca^{2+}-dependent pathways. Importantly, exogenous application of ROS stimulates many of these same cascades. Ligand-induced receptor activation rapidly increases intracellular $O_2^{\cdot-}$ and H_2O_2, and accumulating evidence suggests that these endogenously derived ROS play critical roles as intracellular signaling molecules. The molecular targets of ROS in agonist-stimulated signal transduction have been the subject of intense recent investigation.

The mitogen-activated protein kinase (MAPK) family comprises key regulatory proteins that control the cellular response to growth, apoptosis, and stress signals. In VSMCs, although both H_2O_2 and $O_2^{\cdot-}$ promote cell growth, only $O_2^{\cdot-}$ activates p42/44 MAPKs (extracellular signal–regulated kinase [ERK] 1 and ERK2).[58] Interestingly, angiotensin II, which produces both $O_2^{\cdot-}$ and H_2O_2, potently activates ERK1/ERK2 and p38 MAPK.[59,60] However, only p38 MAPK activation by angiotensin II is sensitive to both inhibition of NAD(P)H oxidase activity and catalase overexpression in VSMCs.[60] In contrast, PDGF-induced ERK1/ERK2 activation is inhibited by exogenously applied catalase.[39] ERK1/ERK2 activation in ECs can also be redox sensitive, as shown by the observation that shear stress–induced ERK1/ERK2 tyrosine phosphorylation is inhibited by antioxidants and negative dominant rac-1.[61] Finally, in perfused rat hearts, JNK and p38 MAPK are activated by H_2O_2 and ischemia reperfusion.[62]

The cell survival kinase Akt (protein kinase B) is another potential redox-sensitive kinase.[63] Both exogenous H_2O_2 and angiotensin II induce Akt activation in VSMCs.[64] Importantly, angiotensin II–induced Akt phosphorylation is inhibited by DPI or overexpression of catalase, indicating a role for the NAD(P)H oxidase in agonist-induced Akt activation. Akt may be involved in the redox-sensitive signaling leading to VSMC hypertrophy, because dominant negative Akt inhibits angiotensin II–stimulated [^{3}H]leucine incorporation in cultured VSMCs.[64]

Other potential redox-sensitive signaling targets include ras/rac,[34,65–67] c-src,[68] protein kinase C,[69] tyrosine phosphatases,[70–72] and regulation of Ca^{2+} signaling (reviewed in References 2 and 73). Our recent data suggest that not only

are the proteins themselves potential targets, but also interactions between molecules within a signaling complex may be modified by ROS (M. Ushio-Fukai, unpublished data, 1999).

Redox-Sensitive Gene Expression

Because of the manifold hormones and growth factors that alter tissue and intracellular levels of ROS and the varied and critical signaling pathways activated by ROS, it is not surprising that many cardiovascular-related genes are redox sensitive. ROS regulate several general classes of genes, including adhesion molecules and chemotactic factors, antioxidant enzymes, and vasoactive substances. Some of these genes clearly provide an adaptive response, such as the induction of superoxide dismutase (SOD) and catalase by H_2O_2.[74] As opposed to being responsive to externally applied oxidant stress or to being regulated by an unknown source of ROS, only a few of these genes have been demonstrated to be downstream of the NAD(P)H oxidases. Arai et al[75] showed that induction of intercellular adhesion molecule-1 by TNF-α was inhibited by multiple antioxidants, including DPI. DPI also blocked lactosylceramide upregulation of intercellular adhesion molecule-1.[40] MCP-1 is another gene that appears to be responsive to NAD(P)H oxidase activation. Oxidase inhibitors attenuate the induction of MCP-1 by angiotensin II[76] and PDGF[38] in VSMCs, as well as by TNF-α in fibroblasts. In contrast, stimulation of MCP-1 by interleukin-1β[38] or by TNF-α[77] in VSMCs is unaffected by antioxidants, suggesting that redox-sensitive control of gene expression is both tissue and stimulus specific.

NAD(P)H Oxidase and Cell Growth

One of the most important functions of NAD(P)H oxidase–derived $O_2^{\cdot-}$ and H_2O_2 in VSMCs and cardiac myocytes is participation in cell growth. In VSMCs, angiotensin II induces cellular hypertrophy by acting through G protein–coupled AT_1 receptors.[78,79] It has recently been demonstrated that angiotensin II–induced hypertrophy is mediated by intracellularly produced H_2O_2 that is derived, at least in part, from a membrane-associated NAD(P)H oxidase.[6,21,30] This conclusion was based on 3 lines of evidence. First, angiotensin II–induced hypertrophy is abrogated by DPI, an inhibitor of NAD(P)H oxidase.[6] Second, reduction of NAD(P)H oxidase activity by transfection of antisense p22phox inhibits both H_2O_2 and hypertrophy, suggesting that H_2O_2 might be the relevant ROS.[30] This was confirmed by the third set of data, namely that catalase overexpression attenuates the angiotensin II–induced effects on growth.[21] Similar results have been found in cardiac myocytes, in which it was shown that angiotensin II– and TNF-α–induced hypertrophy is associated with intracellular release of ROS and that antioxidants blocked both events.[80] Furthermore, in VSMCs, endogenous ROS production is stimulated by PDGF and epidermal growth factor,[39] lactosylceramide (a ceramide analogue present in atherosclerotic plaque),[81] phenylephrine,[82] and thrombin.[19] In all cases, the increases in ROS and proliferation induced by these agonists were shown to be inhibited by antioxidants such as DPI, *N*-acetylcysteine, or catalase.

NAD(P)H Oxidase and Atherosclerosis

The original hypothesis that atherosclerosis is an inflammatory disease centered on oxidized LDL as the main effector. It is clear, however, that oxidative stress plays a much broader role in the pathogenesis of this disease. Animal models of atherosclerosis have documented that all of the constituents of the plaque produce and use ROS.[6,7,14,25,83] In cholesterol-fed rabbits, $O_2^{\cdot-}$ is increased in the aorta,[26] and treatment with polyethyleneglycol SOD reverses the impaired endothelial-dependent relaxation.[53]

Recently, in a rabbit model of early atherosclerosis (Watanabe rabbits, in which hypercholesterolemia is secondary to a LDL-receptor defect), Warnholtz et al[84] showed that vascular NADH-driven $O_2^{\cdot-}$ was increased 2-fold compared with control after 8 weeks. Endothelial denudation normalized the differences in $O_2^{\cdot-}$, suggesting that the excess $O_2^{\cdot-}$ production was localized to the intima. Interestingly, treatment with the AT_1 receptor antagonist BAY 10-6734 normalized $O_2^{\cdot-}$ and endothelial function and reduced early atherosclerotic lesion formation, suggesting a role for angiotensin II in this hypercholesterolemic model of early atherosclerosis.

In a chronic, advanced model of atherosclerosis (2 to 4 years of hypercholesterolemia), Miller et al[85] found that aortas from Watanabe rabbits had $O_2^{\cdot-}$ levels 3 times higher than rabbits with normal levels of cholesterol. In contrast to the previous study, the correction of endothelial $O_2^{\cdot-}$ production with adenoviruses encoding Cu/Zn SOD or extracellular SOD did not improve endothelium-dependent relaxation.[85] These data suggest that the cellular source and functional consequence of $O_2^{\cdot-}$ production may evolve in a spatiotemporal manner from the endothelium toward the medial-adventitial side of the vessel wall. This process bears a striking similarity to the spatiotemporal evolution of atherosclerosis.

In more advanced atherosclerotic lesions, VSMC NAD(P)H oxidase-derived ROS may play a crucial role in progression and biological activity. Angiotensin II, PDGF, and TNF-α may increase ROS in the atherosclerotic lesion by stimulating the local vascular myocytes to produce ROS, as they do in culture. Subsequently, ROS may contribute to LDL oxidation,[54] local MCP-1 production,[38,76] upregulation of adhesion molecules and macrophage recruitment,[55,75] endothelial dysfunction,[53] and extracellular matrix remodeling through collagen degradation and eventually plaque rupture.[56,86,87]

NAD(P)H Oxidase and Hypertension

Compelling evidence has accumulated to support a role for $O_2^{\cdot-}$ in various forms of hypertension. In a spontaneously hypertensive rat model, Suzuki et al[88] showed that $O_2^{\cdot-}$ is increased in venules and arterioles, and Nakazono et al[89] provided evidence confirming the functional importance of ROS by demonstrating that administration of heparin-binding SOD, which localizes within the vessel wall, normalized the blood pressure of these rats. Rajagopalan et

al[8] and Fukui et al[90] demonstrated that chronic infusion of angiotensin II in rats upregulates vascular p22phox mRNA and increases NAD(P)H oxidase-derived $O_2^{\cdot-}$. Both the hypertension and the increase in p22phox mRNA were prevented by pretreatment with SOD.[49,91] Hypertension was also correlated with high oxidative stress in Dahl hypertensive rats.[92] In contrast, norepinephrine-induced hypertension increases neither $O_2^{\cdot-}$ production nor oxidase expression,[8] suggesting that $O_2^{\cdot-}$ is involved in only certain forms of hypertension.

Future Directions

Although much progress has been made in identifying and characterizing the NAD(P)H oxidases, significant work remains. Further information concerning the molecular structure and activation mechanisms of the oxidase in different cardiovascular cells is needed. In addition, identification of the enzymatic and protein targets of oxidase-derived ROS should be a priority. The ultimate goal, of course, is the development of new therapeutic strategies for the treatment of the multitude of cardiovascular diseases of which the pathology derives in part or in total from increased oxidative stress. Understanding the molecular mechanisms leading to generation of ROS and the endogenous antioxidant enzymes responsible for their removal should provide new targets for the emerging therapeutic paradigm of gene therapy.

Acknowledgments

This review was supported by NIH Grants HL38206, HL58000, and HL58863. We thank Carolyn Morris for excellent secretarial assistance.

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