Subcellular Localization of Oxidants and Redox Modulation of Endothelial Nitric Oxide Synthase

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Reactive oxygen species (ROS) have long been viewed as deleterious chemicals that lead to oxidative stress. More recently, ROS, especially the stable ROS hydrogen peroxide (H₂O₂), have been shown to have roles in normal physiological responses in vascular cells. Endothelial nitric oxide synthase (eNOS) is dynamically targeted to plasmalemmal caveolae, and represents the principal enzymatic source of nitric oxide (NO) in the vascular wall. eNOS maintains normal vascular tone and inhibits the clinical expression of many cardiovascular diseases. Increases in oxidative stress are associated with eNOS dysfunction. In a paradigm shift in the conceptual framework linking redox biochemistry and vascular function, H₂O₂ has been established as a physiological mediator in signaling pathways, yet the intracellular sources of H₂O₂ and their regulation remain incompletely understood. The subcellular distributions of ROS and of ROS-modified proteins critically influence the redox-sensitive regulation of eNOS-dependent pathways. ROS localization in specific subcellular compartments can lead to selective oxidative modifications of eNOS and eNOS-associated proteins. Likewise, the dynamic targeting of eNOS and other signaling proteins influences their interactions with reactive nitrogen species and ROS that are also differentially distributed within the cell. Thus, the subcellular distribution both of eNOS and redox-active biomolecules serves as a critical basis for the control of the “redox switch” that influences NO- and oxidant-regulated signaling pathways. Here we discuss the biochemical factors, cellular determinants, and molecular mechanisms that modulate redox-sensitive regulation of eNOS and NO- signaling under normal and pathological conditions. (Circ J 2012; 76: 2497–2512)

Key Words: Nitric oxide; Nitric oxide synthase; Reactive oxygen species

Pathological perturbations of the intracellular redox potential affecting nitric oxide (NO·)-dependent cell signaling pathways in vascular endothelial and smooth muscle cells can lead to the development of vascular dysfunction across a broad spectrum of cardiopulmonary and cardiovascular diseases. The association of oxidative stress with chronic disease states ranging from atherosclerosis to neurodegeneration has led to numerous studies exploring the effects of antioxidant vitamins and dietary supplements. Although some clinical trials have found beneficial effects of these, meta-analyses of numerous clinical trials have found no net benefit, and there may possibly be detrimental effects of nonspecific antioxidant therapies. These unsuccessful clinical efforts to use antioxidant vitamins or dietary supplements to prevent or ameliorate the detrimental effects of increased vascular oxidant stress in humans have led to a resurgence of investigations that aim to define with greater specificity the precise mechanism(s) by which oxygen free radicals promote vascular diseases. The recognition of physiological roles for reactive oxygen species (ROS) such as hydrogen peroxide (H₂O₂) may indicate that the broad use of nonspecific antioxidant supple-
tation of eNOS is dynamically regulated and the enzyme is thus exposed to different concentrations of ROS depending upon where in the cell the protein is localized. Alterations in eNOS function due to changes in ROS levels may reflect either physiological or pathological responses in both normal cells and in vascular disease states. The relationship between impaired eNOS activity and the development of vascular dysfunction has been extensively studied: NO is a potent vasodilator molecule that also attenuates platelet aggregation, vascular smooth muscle cell (VSMC) proliferation, and negative vascular remodeling. Collectively, these properties contribute to the prevention of intermediate pathophenotypes of vascular disease, including cardiac and/or vascular hypertrophy, fibrosis, and atherosclerosis. In counterpoise to these deleterious effects of ROS, there also appears to be a key physiological role for H₂O₂ in eNOS regulation. Elucidating the redox-dependent mechanisms involved in the physiological and pathophysiological regulation of eNOS by ROS will provide critical new insights into vascular disease states and may lead to the identification of novel treatment targets related to eNOS and eNOS-modulated vascular responses.

The Intracellular Redox Potential: Subcellular Localization of Oxidants to Modulate Vascular Function

Vascular endothelial and smooth muscle cells normally maintain a net reductive electrochemical potential in the intracellular space, despite numerous environmental, dietary, and other acquired factors that challenge this homeostasis through increased ROS levels. Importantly, distribution of the intracellular redox potential is inhomogeneous: specific subcellular compartments have different reductive environments leading to distinct patterns of protein oxidative modifications in disparate regions of the cell. One proposed hierarchy of redox potential in eukaryotic cell structures is as follows: [most oxidizing] mitochondrion> nucleus> cytoplasm> endoplasmic reticulum> extracellular [most reductive]. The biological importance of these differences in redox potential has direct implications for cellular signaling; for example, the local redox milieu and hydrophobicity of the cell membrane favors the formation of S-nitrosothiols. Indeed, many key oxidants and nitrosylating agents are non-polar, and may interact freely within the hydrophobic membrane lipid bilayer and be concentrated in biological membranes. This fact has direct implications for eNOS, which is dynamically targeted to plasma membrane caveolae. Caveolae are membrane invaginations that have a distinct lipid composition consisting of sphingolipids, glycosphingolipids, and cholesterol, while being relative depleted of phospholipids. It is not yet fully understood if and how the distinct lipid composition of caveolae affects the distribution and chemical properties of reactive nitrogen and ROS. Moreover, caveolae contain the scaffolding/regulatory protein, caveolin, a transmembrane protein that interacts with and modulates the activity of diverse signaling proteins, including eNOS. It is plausible that the unique protein and lipid environment in caveolae might play a role in modulating the oxidative and nitrosative modifications of signaling molecules that are targeted to this distinct subcellular organelle.

The development of highly specific and sensitive chemical sensors and biosensors have enhanced the specificity and sen-
sitivity whereby ROS flux can be measured in individual subcellular compartments. These advances have identified intramitochondrial compartments that are responsible for ROS generation under specific (patho)biological conditions, and have also documented NOX translocation as a determinant of spatially-restricted ROS generation. In addition to the spatial heterogeneity of ROS synthesis, it is important to note that pathways involved in ROS catabolism may also be differentially targeted within cells: it is not sufficient to consider only ROS synthesis without also analyzing ROS degradation. Taken together, these novel discoveries have translated into the identification of new mechanisms that link acquired/environmental stimulators of oxidant stress with changes in vascular function. For example, Waypa et al used ratiometric fluorescent protein biosensor methods to demonstrate intramitochondrial compartment-specific changes in ROS levels within pulmonary artery smooth muscle cells under hypoxic conditions. Cell exposure to hypoxia leads to an increase in ROS levels within the mitochondrial intramembrane space, but not within the mitochondrial matrix, possibly representing a redox-sensitive mechanism controlling hypoxic pulmonary vasocostriction.

A growing body of evidence supports the hypothesis that the subcellular compartmentalization of redox-active molecules can critically regulate eNOS itself. For example, eNOS targeting plasmalemmal caveolae is a key determinant of its S-nitrosylation, which serves to deactivate the enzyme (see later section on S-nitrosylation of eNOS). By contrast, the reductive potential of the cytosol favors eNOS denitrosylation and enhances enzyme activation. Whereas wild-type caveolae-targeted eNOS is robustly S-nitrosylated, the acylation-deficient eNOS mutant (Myr<sup>−</sup>), which is expressed exclusively in the cytosol, fails entirely to undergo this modification (Figure IA). Similarly, wild-type eNOS undergoes sulfenation, whereas the Myr<sup>−</sup> mutant cystolic eNOS shows no sulfenation whatsoever (Figure IB). Thus, two critical redox-modulated post-translational modifications of eNOS are entirely dependent on the enzyme’s subcellular localization. Because eNOS undergoes dynamic agonist modulated translocation (see review by Dudzinski et al<sup>15</sup>), the marked differences in the subcellular distribution of redox potential may exert a significant effect on eNOS-dependent signaling responses. In addition to differences in the local redox potential between the cell membrane and the cytosol, changes to the 3-dimensional structure of target proteins in various intracellular compartments and relative abundances of enzymes involved in S-nitrosylation are postulated as contributing factors in the differential activation of eNOS.<sup>19,21</sup>

### ROS and Factors Influencing Their Subcellular Localization

The term “reactive oxygen species” describes a group of molecules derived from O<sub>2</sub> (formed from molecular oxygen) and synthesized through a series of 1-electron reduction reactions. For example, superoxide anion may be reduced spontaneously or dismutated by superoxide dismutase (SOD) to form the weaker oxidant H<sub>2</sub>O<sub>2</sub>. In turn, proton abstraction by xanthine oxidase-generated O<sub>2</sub>: results in the formation of the strong oxidant perhydroxyl radical (HO<sub>2</sub>•), which may initiate fatty acid (LOOH) peroxidation in a process implicated in the pathobiology of atherogenesis and atherothrombosis. Importantly, each specific ROS is associated with a unique biochemical profile, oxidizing potential, capacity to induce 1- or 2-electron oxidation reactions, and propensity for reduction in the presence of antioxidants (Table). Key enzymatic sources of O<sub>2</sub>: generation include the electron transport chain in mitochondria, xanthine/xanthine oxidase, NOX (the NOX type-4 enzyme isoform may catalyze the 2-electron reduction of oxygen to directly synthesize H<sub>2</sub>O<sub>2</sub>), and uncoupled eNOS (reviewed by Sugamura and Keaney<sup>22</sup>) (Figure 2).

The net charge of nitrosylating agents and some oxidants (eg, H<sub>2</sub>O<sub>2</sub>) is a key factor in determining the subcellular compartmentalization of S-nitrosylation and post-translational protein oxidative modifications. For example, H<sub>2</sub>O<sub>2</sub> is non-polar and lipophilic; these biochemical properties may facilitate the concentration of H<sub>2</sub>O<sub>2</sub> in cell membrane-bound structures and/or permit H<sub>2</sub>O<sub>2</sub> to traverse the cell membrane to transduce intercellular signaling functions. Another key biochemical factor that contributes to subcellular localization is the proclivity of H<sub>2</sub>O<sub>2</sub> to rapidly interact with heme-containing proteins and thiolates (R-SH<sup>−</sup>). These rapid reaction rates, which are determined in vitro in solution, support observations of the interaction of H<sub>2</sub>O<sub>2</sub> with various heme-containing proteins and cysteinyl thiol(s) important in modulating eNOS-dependent cell signaling, including eNOS, soluble guanylyl cyclase.

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**Table. Reactivity of Selected Biologically Important Oxidants**

<table>
<thead>
<tr>
<th>Radical Name</th>
<th>1- or 2-electron oxidant</th>
<th>Reduction potential (1 electron) (V) at pH 7</th>
<th>Rate constant (M/s) with GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>O&lt;sub&gt;2&lt;/sub&gt;-</td>
<td>Superoxide anion</td>
<td>1</td>
<td>6.7x10&lt;sup&gt;8&lt;/sup&gt;•</td>
</tr>
<tr>
<td>OH&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Hydroxyl radical</td>
<td>1</td>
<td>2.31</td>
</tr>
<tr>
<td>Cys&lt;sup&gt;-&lt;/sup&gt;/Cys&lt;sup&gt;-&lt;/sup&gt;S&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Cysteine/cystine</td>
<td>1</td>
<td>0.92</td>
</tr>
<tr>
<td>RO&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Alkoyl</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>NO&lt;sub&gt;2&lt;/sub&gt;-</td>
<td>Nitrogen dioxide</td>
<td>1</td>
<td>1.04</td>
</tr>
<tr>
<td>HOCl</td>
<td>Hypochlorous acid</td>
<td>2</td>
<td>3x10&lt;sup&gt;7&lt;/sup&gt;•</td>
</tr>
<tr>
<td>ONOO&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Peroxynitrite anion</td>
<td>2</td>
<td>700**•</td>
</tr>
<tr>
<td>O&lt;sub&gt;2&lt;/sub&gt;NO&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Peroxynitrate anion</td>
<td>2</td>
<td>3x10&lt;sup&gt;4&lt;/sup&gt;•</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Hydrogen peroxide</td>
<td>2</td>
<td>0.9</td>
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<tr>
<td>ROO&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Peroxyl radical</td>
<td>1</td>
<td>0.77–1.44</td>
</tr>
<tr>
<td>NO&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Nitric oxide</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Reduction potentials (E<sub>r</sub>) and reaction rates with reduced GSH are presented for various radicals and oxidants implicated directly or indirectly in the redox regulation of endothelial nitric oxide synthase. These data (adapted from Winterbourn<sup>21</sup>) outline the wide variation in oxidant potential; for example, superoxide anion (O<sub>2</sub>-) is 10<sup>6</sup>-fold more reactive than hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Derived from Huie and Padmaja.<sup>19</sup> **Derived from Gupta et al with permission.<sup>22</sup>

GSH, glutathione.
(sGC), glutathione, thioredoxin, vascular endothelial growth factor receptor-2 (VEGFR-2), endothelin type-B receptor (ETB), and peridorexin, among others. Importantly, several of these potential H₂O₂ targets are localized in cellular membranes, where the hydrophobic environment may facilitate the interaction of these proteins with H₂O₂.

Other subcellular locales have been studied in the context of site-specific targeting by oxidant stress. Mitochondrial ROS flux is complexly determined, reflecting the intricate network of enzymes regulating the metabolism of oxidants, including the electron transport chain, NOX-4, glutathione peroxidase-1, peroxiredoxins (see later), thioredoxin, and SOD. Although eNOS does not appear to localize to mitochondria, cross-talk between pathways that regulate both mitochondrial bioenergetics and eNOS has been described through the Silent mating type information regulation homolog (SIRT1) protein (see later section on the structure and function of eNOS). SIRT1 upregulation in mitochondria occurs at the expense of increased levels of the oxidizing agent NAD⁺, which contributes to enhanced mitochondrial respiration. The downstream consequences of increased mitochondrial ROS flux with respect to SIRT-1-eNOS signaling are not fully resolved.

**Figure 2.** Key enzymatic sources of reactive oxygen species (ROS). Mitochondrial complexes I and III are important components of the electron transport chain in mammalian mitochondria that are involved in the generation of ROS. Under physiological conditions, the vast majority of all electrons that pass through this system reach the final step to react with molecular oxygen and participate in the synthesis of water. However, under pathological conditions, incomplete flow of electrons results in the preferential formation of superoxide anion (•O₂⁻) to water. Superoxide may be dismutated to hydrogen peroxide (H₂O₂) and, subsequently, to the hydroxyl radical (•OH). Importantly, H₂O₂ is able to diffuse freely across the inner/outer mitochondrial membrane (dotted line), whereas •O₂⁻ flux into the intracellular compartment is receptor-dependent. Additional important enzymatic sources of intracellular ROS generation include lipooxygenases, uncoupled eNOS, xanthine oxidase and the multimeric protein complex NADPH oxidase (NOX). I, II, III, IV are the different mitochondrial complexes. NADPH, nicotinamide adenine dinucleotide phosphate; NAD, nicotinamide adenine dinucleotide; AA, arachidonic acid; HETE, hydroxyeicosatetraenoic acid; HPETE, 5-hydroperoxyeicosatetraenoic acid; SOD, superoxide dismutase.

**Subcellular Localization of Oxidants in a Reductive Environment**

A long-standing puzzle in redox biochemistry and human disease has been the observation that oxidants, particularly H₂O₂, may regulate protein function despite the intrinsically reductive redox state of the cell and an abundance of H₂O₂-detoxifying enzymes such as catalase and peroxiredoxin. Woo et al demonstrated that NOX-derived H₂O₂ generation induces phosphorylation of (membrane-bound) peroxiredoxin I (PrxI) through a mechanism involving Src, which phosphorylates and deactivates PrxI, thereby promoting the local accumulation of bioactive H₂O₂. These elegant observations provide support to linking H₂O₂ and vascular disease despite normal antioxidant defenses: redox-dependent signal transduction may be governed by local regulatory mechanisms that offset the tendency for vascular cells to detoxify peroxides, thus enabling oxidant-mediated signaling.

**eNOS and NO⁺ Under (Patho)physiological Conditions**

**Structure and Function of eNOS**

NO is synthesized in endothelial cells by eNOS, a homodimeric enzyme containing an N-terminal heme-binding (oxygenase) domain and a C-terminal reductase domain (Figure 3).
Redox Regulation of eNOS

The reductase domain is structurally similar to cytochrome P450 reductase; however, NO synthases have the distinctive property of the oxygenase and reductase domains being separated by a calmodulin-binding region. The oxygenase domain of eNOS contains binding sites for heme, tetrahydrobiopterin (BH$_4$), and the substrate L-arginine.\textsuperscript{9} The C-terminal flavoprotein oxidoreductase domain binds flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and NADPH. Under basal conditions, electron transfer between NOS domains is suppressed; however, in the setting of increased intracellular [Ca$^{2+}$], the formation of a Ca$^{2+}$/calmodulin complex disrupts eNOS suppression from the eNOS-caveolin interaction, thereby permitting the transfer of electrons from the reductase to the oxygenase domains of eNOS.\textsuperscript{41} The oxidation of the guanidine nitrogen of L-arginine is catalyzed by eNOS, which proceeds in 2 distinct monoxygenation steps, each involving electron transfer to molecular oxygen, ultimately resulting in the release of 1 mole each of NO and L-citrulline.\textsuperscript{41}

**Nitric Oxide Biology**

Nitric oxide is a small (30kDa) gaseous and lipophilic molecule, key properties that enable its function as a juxtacrine mediator of cell signaling processes that modulate blood vessel relaxation.\textsuperscript{42-44} These characteristics contribute to the diffusion-limited movement of NO$^\bullet$ between vascular endothelial and smooth muscle cells,\textsuperscript{45} which favors rapid intercellular signaling that is not regulated by the kinetic constraints of receptor-mediated signal transduction. The free radical structure of NO$^\bullet$ affords its interaction with biomolecules, including thiols (R-SH), metals (ie, Fe$^{2+}$), and other free radicals such as superoxide anion ($\bullet$O$_2^{-}$).\textsuperscript{45,46} The range of NO$^\bullet$ molecular targets reflect the versatile biochemical framework that governs NO$^\bullet$ biology. For example, protein cysteine-NO$^\bullet$ interactions form nitrosothiols adducts, which offset the rapid degradation of NO$^\bullet$ and allow NO$^\bullet$ to target remote sites; the interaction of NO$^\bullet$ with the heme ligand of sGC functions as the principle mechanism underlying vascular relaxation; and the formation of peroxynitrite anion (ONOO$^-$) through the rapid interaction of NO$^\bullet$ with $\bullet$O$_2^{-}$ functions as a key redox-sensitive counter-regulatory mechanism that may serve to limit the bioavailability of NO$^\bullet$.

![Molecular structure of endothelial nitric oxide synthase (eNOS).](image-url)
Functions of NO* in Vascular Tissue

NO* has been implicated in a number of processes that are integral to maintaining normal blood vessel structure and function.\(^{48,49}\) Chief among these is cGMP-dependent protein kinase G-1α (PKG-1α)-dependent VSMC relaxation that occurs in response to activation of sGC by NO*. Low concentrations of NO* are sufficient to increase sGC activity ~30-fold, increasing cyclic guanosine monophosphate (cGMP), which activates PKG-1, leading to decreased intracellular calcium and vascular relaxation.\(^{50}\) Alternatively, NO* may modulate vascular tone by targeting Ca\(^{2+}\)-dependent potassium channels directly to attenuate [Ca\(^{2+}\)]\(_{i}\) flux.\(^{51}\) NO* decreases vascular endothelial expression of adhesion molecules, such as ICAM-1,\(^{52}\) P-selectin,\(^{53}\) and \(\beta-1\) integrin,\(^{54}\) which are implicated in cellular migration, proliferation, atherogenesis, and arterial/venous thrombosis.\(^{55}\) NO* exerts pleiotropic antithrombotic effects by (1) increasing sGC-derived cGMP to decrease the [Ca\(^{2+}\)]\(_{i}\) flux that is required for activation of the platelet aggregation molecule glycoprotein IIb/IIIa,\(^{56}\) (2) inhibiting thrombin,\(^{57}\) (3) increasing synthesis of prostacyclin,\(^{58}\) and (4) increasing cAMP levels to induce phospholamban phosphorylation-mediated decreases in [Ca\(^{2+}\)].\(^{59}\) (Figure 4).

NO* may also indirectly influence blood vessel tone by functioning as an antioxidant inhibiting the effects of ROS on vasodilatory cell signaling pathways; for example, through NO*-mediated upregulation of heme-oxygenase-I (see later section on antioxidant enzymes) and SOD.\(^{60}\) The dismutation of the strong oxidant superoxide •O\(^2-\) to H\(_2\)O\(_2\), which is a weaker oxidant, may also lead to activation of eNOS via the oxidation of redox-sensitive protein kinases that promote eNOS signaling.\(^{61}\) Arrows and circular heads indicate agonist and inhibitory effects, respectively. PKG, cGMP-dependent protein kinase G-1α. (Adapted with modifications from Maron and Michel with permission.)\(^{172}\)

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**Figure 4.** Pleiotropic effects of endothelial nitric oxide synthase-derived nitric oxide in vascular tissue. Endothelial nitric oxide synthase (eNOS) is the principal enzymatic source of nitric oxide (NO*) in vascular cells. Activation of eNOS occurs in response to acetylcholine, shear stress or via receptor-mediated activation by endothelin-1 (ET), vascular endothelial growth factor (VEGF), or bradykinin. NO* derived from eNOS or pharmacological NO* donors diffuses across the platelet or vascular smooth muscle cell (VSMC) membrane, resulting in activation of the heterodimeric soluble guanylyl cyclase (sGC). Normal NO*-sGC signaling results in a robust increase in the synthesis of cGMP, which functions as a second messenger to stimulate protein kinase G (PKG)-dependent activation of myosin light chain phosphatase (MLCP) and promotes VSMC relaxation and inhibits platelet reactivity in platelets. Alternatively, cGMP may modulate VSMC relaxation by decreasing intracellular levels of Ca\(^{2+}\) via cGMP-induced inhibition of Ca\(^{2+}\) mobilization through L-type Ca\(^{2+}\) channels; through stimulation of the inositol triphosphate-3 receptor (IP\(_3\)-R); or by decreasing intracellular Ca\(^{2+}\) levels directly via activation of Ca\(^{2+}\)-dependent K\(^+\) exchange, Na\(^{+}\)-Ca\(^{2+}\) exchange, Ca\(^{2+}\)-Mg\(^{2+}\) ATPase(s), and phospholamban (PL) in the sarcoplasmic reticulum (SR). Collectively, these changes mediated by NO* modulate normal vascular tone by inducing blood vessel relaxation, inhibiting vascular cellular proliferation, and inhibiting vascular fibrosis. In turn, elevated levels of reactive oxygen species (ROS) may affect adversely normal eNOS-NO* signaling by oxidizing functional cysteines in the ET-B receptor, VEGF receptor (VEGFR2), eNOS, sGC, and/or PKG. Arrows and circular heads indicate agonist and inhibitory effects, respectively. PKG, cGMP-dependent protein kinase G-1α. (Adapted with modifications from Maron and Michel with permission.)\(^{172}\)
Nitric Oxide and the Formation of S-Nitrosothiols

By functioning as a molecular adduct for NO\(^{−}\), the formation of S-nitrosothiols was originally proposed in order to account for the observed difference between the half-life of NO\(^{−}\) in vitro (0.1 s) compared with its half-life in vivo (~6 s).\(^{63}\) Early experiments studied the cysteine-rich structure of human serum albumin, which contains 17 disulfide bonds and a free cysteine at position Cys34 that has an anomalously low pKa, and is therefore highly reactive, to demonstrate that human plasma contains 7 \(\mu\)M S-nitrosothiols, of which approximately 80% are accounted for by S-nitroso-serum albumin.\(^{64}\) The functional consequences of protein S-nitrosylation are recognized as being implicated in many aspects of cardiovascular (patho)physiology, including atherogenesis, regulation of NOS activity (see later), electrophysiological signaling, myocyte contractility, formation of nitrosohemoglobin, regulation of mitochondrial bioenergetics, and the modulation of hypoxic pulmonary vasoconstriction (reviewed by Maron et al\(^{65}\)).

Although the biochemistry of protein S-nitrosylation is complex, it is clear that NO\(^{−}\) does not spontaneously bind to thiols under physiological conditions (Figure 5). S-nitrosylation occurs through liberation of the nitrosonium ion (NO\(^{+}\)) to covalently bind thiols during the metabolism of reactive nitrogen oxides such as N\(_2\)O\(_3\), which is a strong nitrosating agent.\(^{66}\) The predilection for NO\(^{−}\) to participate in bonding with thiols depends on the relative nucleophilicity of thiol functionalities, with the thiolate ion (R-S\(^{−}\)) serving as a strong nucleophile and, thus biochemically favorable for interaction with NO\(^{−}\) to form S-nitrosothiols.\(^{67}\) Importantly, the distribution of nitrosylating agents within the intracellular compartments is indicative of each agent’s biochemical properties. For example, the nitrosylating agent N\(_2\)O\(_3\), which is lipophilic and known to form within protein-hydrophobic cores,\(^{68}\) tends to localize to the lipid bilayer of cell membranes. This pattern of N\(_2\)O\(_3\) subcellular distribution is consistent with that observed for other lipophilic nitrosylating agents and is a factor that favors the cell membrane as a site of S-nitrosylation.\(^{13,19,69}\)

Role of eNOS and NO\(^{−}\) Deficiencies in Human Disease

Deficient bioavailable/bioactive levels of NO\(^{−}\) are associated with the development of important human vascular diseases, including myocardial infarction, stroke, essential hypertension, and pulmonary hypertension.\(^{1}\) Analyses of genetically engineered animals deficient in eNOS expression (eNOS\(^{−/−}\)) provide support for the importance of eNOS-derived NO in vascular homeostasis. For example, eNOS\(^{−/−}\) mice, which lack eNOS-derived NO\(^{−}\), develop numerous vascular disease states, including systemic hypertension, increased leukocyte-endothelial adhesion,\(^{78}\) platelet aggregation and thrombosis,\(^{71}\) atherogenesis,\(^{72}\) and abnormal pulmonary vascular reactivity.\(^{73}\)

Compensatory upregulation of the neuronal/inducible NOS isoforms has been proposed as a mechanism to account for detectable levels of bioavailable NO\(^{−}\) in eNOS\(^{−/−}\) mice. At low pH, dietary nitrite (NO\(^{−}\)) derived from nitrate (NO\(^{3−}\)) synthesized by commensal bacteria may be converted to nitrous acid (HNO\(_2\)) and spontaneously form NO\(^{−}\) (Figure 5).
Redox Regulation of eNOS: Enzyme Post-Translational Modifications

Changes to the local redox potential may affect eNOS-dependent NO synthesis in several ways. First, ROS, particularly •O₂⁻, may oxidize BH₄ to form BH₂, which is unable to serve as an eNOS cofactor. As a result, eNOS “uncoupling” occurs, resulting in the preferential formation of •O₂⁻ (and a peroxyl radical of BH₄) (Figure 3A). eNOS uncoupling is also associated with decreased L-arginine and/or accumulation of methyarginines, which affects the balance of eNOS-derived •O₂⁻/NO synthesis. Increased ROS generation may consume NO; for example, via formation of reactive nitrogen species (RNS) such as ONOO⁻ or peroxynitrite (O₂NOO⁻), synthesized from the interaction of •O₂⁻ and NO₂⁻, which is a substrate for NO synthesis. Additionally, protonation of ONOO⁻ results in the formation of other RNS, including peroxynitritic acid (ONOOH), the conjugate acid of ONOO⁻, which itself is homolyzed to form nitrogen dioxide (NO₂) and hydroxyl radical (OH⁻) or nitronium ion (NO₂⁺) and hydroxide (OH⁻) [OH⁻ + NO₂ ↔ HOONO → OH⁻ + NO₂⁺].

S-Glutathionylation

BH₄ supplementation in the presence of increased ROS modulates only partial restoration of eNOS-dependent NO synthesis activity, suggesting alternative mechanism(s) that link oxidant stress with enzyme activity. The redox status of specific eNOS-thiol(s) may influence the rate of L-arginine conversion to NO’ by eNOS. Differential effects of regulatory thiol(s) on enzyme activation have been described between other NOS isoforms. Recently, 2 cysteine residues located within the eNOS reductase domain were identified as S-glutathionylation targets. These thiols are believed to reside on the surface of the eNOS reductase domain. Molecular modeling suggests that these cysteines are likely to be surrounded by amino acid residues that permit nucleophilic attack by the thiolate anion. S-glutathionylation has been demonstrated to induce eNOS uncoupling (Figure 6). In turn, site-directed mutagenesis substituting cysteine 908 for alanine abolished eNOS thyl radical formation. Oxidant stress generated through the S-glutathionylation reaction is not inhibited by competitive inhibitors of the NOS substrate L-arginine. The relevance of this pathway has been studied in Wistar rats, in which S-glutathionylation is associated with impaired endothelium-dependent vasodilation.

S-Nitrosylation of eNOS

In addition to S-glutathionylation, alternative thiol-dependent eNOS post-translational modifications have been studied to account for the inhibitory effect of NO’ on enzyme activity. It has been established that S-nitrosylation is involved in the dynamic regulation of eNOS. Inhibition of basal eNOS activity is associated with tonic S-nitrosylation at the zinc-tetrahydrothiole cysteine residue Cys101/107, and eNOS stimulation with receptor-dependent agonists results in eNOS denitrosylation and increased eNOS-dependent NO’ synthesis. A shift from dimeric- to monomeric eNOS due to S-nitrosylation may influence enzyme function, although it is not yet clear that dynamic regulation of eNOS dimer formation is a physiologically relevant mechanism for controlling eNOS activity. Other Cys101 eNOS cysteine thiols may also undergo S-nitrosylation in vitro, although the functional consequences for cellular regulation have not been established.

Hydrogen Peroxide-Mediated Redox Regulation of eNOS

The functional effects of H₂O₂ are linked to cell type, H₂O₂ concentration, and treatment conditions. For example, NOX-dependent synthesis of (pathophysiological) H₂O₂ concentra-
Redox Regulation of eNOS

Upstream and Downstream of eNOS Activation

Upstream signaling intermediaries implicated in eNOS activation have been proposed as redox-sensitive targets include bradykinin and caveolae. Here, the mechanistic and functional consequences of perturbations to the intracellular redox status on Akt, the ET receptors, and vascular endothelial growth factor (VEGF) in the context of eNOS activation are discussed.

Akt- and AMPK-Dependent eNOS Phosphorylation

Whereas lower concentrations of H\textsubscript{2}O\textsubscript{2} (<100 \textmu mol/L) are associated with physiological cell signaling, higher H\textsubscript{2}O\textsubscript{2} concentrations (≥500 \textmu mol/L) may suppress eNOS phosphorylation and enzyme activation. The AMP-activated protein kinase (AMPK)-stimulated phosphorylation of eNOS was found to be dependent upon H\textsubscript{2}O\textsubscript{2}-mediated phosphorylation of the upstream protein kinase CaMKK\textbeta. Thus, H\textsubscript{2}O\textsubscript{2}-modulated protein phosphorylation pathways play a key role in the regulation of eNOS activation. In turn, eNOS activity may modulate cellular levels of H\textsubscript{2}O\textsubscript{2}. Studies using the H\textsubscript{2}O\textsubscript{2} biosensor, HyPer, showed that eNOS knockdown led to increased H\textsubscript{2}O\textsubscript{2} and AMPK phosphorylation in endothelial cells. Clearly, there is a close interplay between physiological levels of H\textsubscript{2}O\textsubscript{2} and eNOS-dependent signaling pathways (Figure 7). Higher H\textsubscript{2}O\textsubscript{2} concentrations appear to have broader effects on oxidant-modulated signaling pathways. Higher H\textsubscript{2}O\textsubscript{2} concentrations are associated with increased depletion of free thiols, possibly via the formation of higher oxidative cysteine intermediates and subsequently diminished eNOS phosphorylation. These data suggest a broader role for soluble as well as membrane-bound thiol oxidative post-translational modification(s) as a physiological regulatory mechanism for eNOS phosphorylation.

ET\textalpha Receptor

The ET\textalpha receptor is a heptahelical G-protein coupled receptor containing an intracellular cysteine-rich region near the N-terminus that is involved in normal ET\textalpha signaling. Recently, it has been demonstrated that exposure of cultured human pulmonary artery endothelial cells to oxidant stress results in the oxidative modification of these ET\textalpha cysteinyl thiol(s), resulting in the formation of higher oxidative species of cysteine, including sulfenic acid (R-SOH) (Figure 8). Cells treated with pathophysiological concentrations of aldosterone akin to levels observed in animal models and in humans with pulmonary arterial hypertension induce NOX-4-dependent H\textsubscript{2}O\textsubscript{2} generation in vascular cells. Protein extracts from treated cells were immunoprecipitated using an anti-ET\textalpha antibody. The immunoprecipitates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Coomassie brilliant blue staining to detect NOX-4 and eNOS protein levels.
antibody specific to the region of ETα containing the putative cysteines and immunoblotting was performed using an anti-sulfenic acid (R-SOH) antibody. Site-directed mutagenesis substituting cysteine for alanine at ETα Cys405 (C405A) inhibited disulfide bond formation in the presence of oxidant stress and preserved ETα-dependent activation of eNOS and NO•α•1 synthesis, suggesting that ETα Cys405 is a critical regulatory cysteine involved in ETα-eNOS signaling.

**VEGF**

The major vascular endothelial growth factor target receptor, VEGFR2, is a tyrosine kinase receptor that modulates various functional effects important to vascular biology. Stimulation of VEGFR2 is associated with Akt-dependent phosphorylation of eNOS, whereas monoclonal antibody-mediated inhibition of VEGFR2 is a bona fide strategy by which VEGF-dependent cellular proliferation is inhibited in various forms of cancers. The redox status of VEGFR2 has been studied with respect to eNOS activation. In an experimental model, the effect of cigarette smoke, which contains ROS and RNS, was used to study redox regulation of VEGFR2-eNOS signaling. Cigarette smoke induced vascular ROS generation in mouse lungs, and exposed lung microvascular endothelial cells in culture showed downregulated VEGFR-2 and eNOS levels, and decreased VEGFR-2 phosphorylation. Although RNS/ROS have been shown to interact directly with VEGFR-2 to modulate this effect, further oxidation of receptor tyrosine residues, the mechanism by which this modulates receptor deactivation in vascular cells is not fully resolved.

Targets upstream of VEGFR-2 that modulate the inhibition of oxidant stress on VEGF-eNOS signaling have been identified. For example, inhibition of poly [ADP-ribose] polymerase (PARP-1), which is a DNA base repair enzyme activated in the presence of oxidant stress, is associated with attenuation of H2O2 or ONOO−-mediated inhibition of VEGFR-2 phosphorylation in endothelial cells. In human umbilical vein endothelial cells and rabbit aortas, inhibition of PARP-1 restores VEGF- and Akt- phosphorylation. PARP-1 inhibition restores eNOS activity and endothelial function in blood vessels of apolipoprotein E (ApoE−/−) mice fed a high fat diet to induce atherosclerosis.

**sGC**

sGC is a heterodimeric enzyme (αβ; αββ) that is activated by NO• and results in the conversion of GTP to the second messenger cGMP, which is a potent stimulator of VSMC relaxation. Normal NO• sensing by sGC requires that the prosthetic heme ligand located near His105 on the β-subunit is reduced (Fe2+). In contrast, ROS-mediated oxidation of heme to the ferric (Fe3+) form impairs NO•-induced sGC activation and is implicated as a possible mechanism linking oxidant stress to the development of essential hypertension, pulmonary hypertension, stroke, and myocardial infarction. Activation of heme-oxidized sGC is a contemporary pharmacotherapeutic target in these diseases. A family of novel synthetic compounds derived from 5-substituted-2-furaldehyde-hydrazone, which are believed to compete with oxidized heme for the enzyme-activating pocket of sGC, effectively activates heme-free sGC in vitro and is under study in ongoing clinical trials for the treatment of pulmonary hypertension.

There is evolving evidence to suggest that sGC contains essential redox-sensitive cysteinyl thiols that participate in the regulation of NO•-sGC signaling. The regulation of sGC by the redox status of functional cysteinyl thiol(s) and heme is interrelated and hierarchical: NO•-heme binding kinetics, and, thus, enzyme activity, are enhanced by NO•-binding to regulatory sGC thiols.

**PKG-1α**

PKG-1α is the principal target of cGMP. Activation of the cGMP-PKG-1α axis stimulates the phosphorylation of numerous target proteins that is required for maintaining vascular tone and platelet function, as well as other key cellular processes such as cell division and nucleic acid synthesis. Regulation of PKG-1α dimerization involves Cys42 on each homodimer; oxidation of Cys42 results in activation of PKG-1α via
intermolecular disulfide bridge formation. The change in PKG-1α due to disulfide bridge formation enhances its affinity for biological substrates. Consistent with this are observations demonstrating that PKG-1 is activated by H\textsubscript{2}O\textsubscript{2} or other thiol oxidants in an SGC-free system. Genetically engineered mice containing a substitution cysteine with serine at position 42 of PKG-1α are incapable of PKG-1 dimerization and show decreased response to NO\textsuperscript{+} donors in vivo.

**Antioxidant Enzymes and the Cellular Redox Potential**

Maintenance of the normal intracellular redox status relies upon endogenous antioxidant enzyme defenses. On the other hand, genetic or acquired antioxidant enzymopathies contribute to a disrupted cellular redox balance due to the depletion of intracellular reducing equivalents, which results in the accumulation of oxygen free radicals and their derivative oxidant species.

**Glucose-6-Phosphate Dehydrogenase (G6PD)**

G6PD catalyzes the first and rate-limiting step of the pentose phosphate pathway\textsuperscript{125} in a reaction that requires NADP\textsuperscript{+} and results in the conversion of G-6-P to 6-phosphogluconate and the synthesis of NADPH. In turn, NADPH is (1) a key intracellular reducing equivalent by maintaining glutathione in the reduced form,\textsuperscript{127} (2) a cofactor utilized by eNOS to generate NO\textsuperscript{−},\textsuperscript{128} and (3) a key participant in the maintenance of normal BH\textsubscript{4} levels via the dihydrofolate reductase salvage pathway. Long recognized as the primary mechanism by which to account for oxidative injury in erythrocytes,\textsuperscript{129} impaired G6PD activity has also been linked to a swath of other pathobiological processes, including diminished eNOS expression and activity that results in vascular endothelial dysfunction,\textsuperscript{128} progressive myocardial dysfunction in response to ischemia-reperfusion injury,\textsuperscript{130} pulmonary hypertension,\textsuperscript{131} and renal dysfunction with albuminuria.\textsuperscript{133} Endothelial cell migration and angiogenesis are also G6PD-dependent processes because enzyme deficiency is linked to diminished VEGF-dependent angiogenesis via decreased eNOS phosphorylation.\textsuperscript{127} By contrast, 5-methyltetrahydrofolate is associated with improved endothelial function owing to its ONOO\textsuperscript{−} scavenging effects, which, in human saphenous vein grafts is associated with an increase in the BH\textsubscript{4}/superoxide ratio.\textsuperscript{98}

**Glutathione Peroxidase (Gpxs)**

Gpxs are a family of 4 biologically relevant (Gpx-1–4) selenium-containing enzymes that utilize reduced glutathione, a low-molecular-weight tripeptide, to reduce H\textsubscript{2}O\textsubscript{2} to water, and lipid hydroperoxides to their corresponding alcohols.\textsuperscript{134} Detoxification of peroxides by Gpxs occurs via bidirectional second-order kinetics, and, thus, is a saturation-limited process. The reaction is enabled by the unique properties of selenium, which reacts with peroxide to convert selenol (R-SeH) to sulfenic acid (R-SeOH).\textsuperscript{134,136,137} Next, 2 moles of glutathione (GSH) reduces R-SeOH to form initially the R-SG intermediate and, subsequently, oxidized glutathione (GSSG). Of the Gpx isoforms, Gpx-1 is the most abundant in mammals. Gpx-1 is present in all cells and localizes to the cytosolic, mitochondrial, and peroxisome cellular ultrastructures.\textsuperscript{138,139} Deficiencies in Gpx-1 are associated with increased vascular oxidant stress levels and endothelial dysfunction.\textsuperscript{141} For example, in mice heterozygous for Gpx-1 (Gpx-1+/−), mesenteric arterioles demonstrate increased vascular tone in response to (the eNOS activator) acetylcholine as compared with wild-type mice.\textsuperscript{142} Interestingly, however, other reports have demonstrated that Gpx-1 levels are elevated in rodent models of hypertension in vivo. Increased Gpx-1 mRNA and protein expression levels are observed in phenylephrine pre-contracted aortic rings of spontaneously hypertensive rats, which is associated with impaired endothelium-dependent vascular relaxation despite attendant elevations in eNOS protein expression and activity level. Authors of that study implicate consumption of NO\textsuperscript{−} by •O\textsuperscript{2−} rather than via direct inhibition of eNOS per se as the mechanism by which to account for these findings. Collectively, these observations illustrate the complexities inherent in anticipating the (pathological) effect(s) of vascular oxidant stress on eNOS—NO\textsuperscript{−} signaling; viz., vessel type-specific properties (ie, systemic resistance or conduit vessels, pulmonary arterioles, etc) and other unpredictable factors that influence reaction kinetics upstream/downstream of eNOS complicate a priori predictions for determining the precise extent to which ROS affects eNOS-dependent signaling.

**Glutathione Reductase (GR) and Thioredoxin Reductases**

The NADPH-dependent enzyme GR reduces oxidized glutathione (GSSG) to reduced glutathione (GSH), which, in turn, is required for Gpx-dependent reduction of peroxides (as discussed above).\textsuperscript{144,145} This is accomplished through the formation of a FAD-bound homodimer in GSR, which results in the generation of the FADH\textsuperscript{−} anion that, in turn, reduces (or 'breaks') the GSSG Cys58-Cys63 disulfide bridge.\textsuperscript{146} This allows for subsequent nucleophile attack of the newly formed thiolate ion (R-SH) to generate a mixed disulfide bond, and, ultimately, 2 GSH molecules. Akin to GR, thioredoxin reductase (Trx) is also a flavin thiol-disulfide oxidoreductase. Trx maintains the intracellular thiol redox status by reducing target thiols disulfides through the reduction of oxidized thioredoxin, at the expense of NADPH-derived electrons.\textsuperscript{147} The family of peroxiredoxins that catalyze the reduction of hydroperoxides belongs to the larger thioredoxin superfamily. The connections between subcellular localization of thiol-metabolizing proteins and physiological cell signaling is exemplified by a recent study in which the phosphorylation of peroxiredoxin-1 resulted in the enzyme inactivation and local accumulation of H\textsubscript{2}O\textsubscript{2}.\textsuperscript{17} These physiological roles of H\textsubscript{2}O\textsubscript{2} must be contrasted with the effects of pathological levels of ROS, which may serve to deplete thiol-based antioxidants in vascular endothelial cells, associated with eNOS dysfunction, cellular senescence, and apoptosis.\textsuperscript{29,148}

Because of the importance of ROS in modulating eNOS activity, the relationship between GR/Trx and eNOS is a target of contemporary scientific investigations. In cultured aortic endothelial cells, pharmacological or molecular inhibition (with siRNA) of GR, TrxR-1, or mitochondrial TrxR-2 is associated with diminished bioavailable NO\textsuperscript{−} because of a decrease in the ratio of reduced-to-oxidized glutathione, VEGF stimulation of the eNOS activator Akt, and eNOS phosphorylation at Ser1179.\textsuperscript{29} Interestingly, in that study, TrxR-2, which is localized primarily to the mitochondrial matrix, but not TrxR-1 or GR inhibition, was associated with a robust increase in H\textsubscript{2}O\textsubscript{2} generation that was not abrogated by BH\textsubscript{4} supplementation. This observation illustrates the differential downstream effects on phosphorylation signaling pathways of redox potential changes associated with Trx-1, TrxR-2, and/or GR, and raises speculation that a hierarchical order by which mitochondrial thiol oxidoreductase functions is most likely among these enzymes in order to modulate eNOS activation.
Protein Disulfide Isomerase (PDI)

PDI is present on the cell surface and catalyzes the thio-disulfide exchange reaction, which, for example, facilitates via trans-S-nitrosylation the traversing of exogenous S-nitroproteins (ie, GSNO) into the intracellular compartment. The functional effects of PDI on eNOS signaling remain elusive, but probably involve interactions with metal binding proteins. For example, metallothionein is a small (7kDa) cysteine-rich protein that functions as an antioxidant and intracellular buffer against the toxic effects of non-essential metals. In the setting of (1) PDI-facilitated entry of NO into the intracellular compartment or (2) rapid increases in [Ca2+], flux resulting in increased levels of bioavailable NO, metallothionein is targeted by NO (and Fe2+) to generate iron nitrosyl thiolates as detected by electron spin resonance (with unique g value signaling of 2.013 and 2.039, respectively). This is postulated to occur via S-transnitrosation that induces the release of metallothionein-containing metals, including cadmium and zinc. Overall, these findings provide a potential mechanism by which to account for the protective effects of metallothionein against eNOS-derived nitrosative stress in some vascular (and non-vascular) cell lines.

Heme Oxygenase

Heme oxygenase is a 32-kDa enzyme that exists as inducible (HO-1) and constitutive isomers (HO-2). Heme oxygenase is the first and rate-limiting step in the metabolism of heme into equimolar concentrations of carbon monoxide, Fe2+, and biliverdin in a reaction that requires molecular oxygen and NADPH. Free intracellular Fe2+ upregulates the expression of heavy-chain ferritin and Fe2+-dependent channels, whereas biliverdin is subsequently reduced by biliverdin reductase to form bilirubin, a non-oxidizable biliverdin. Thus, it follows that HO-1 is a key molecular player in the prevention of lipid peroxidation. Bilirubin also exerts vascular-protective effects by attenuating apoptosis and cellular proliferation. In support of this claim are investigations involving translational animal models of atherosclerosis, which have linked deficient HO-1 activation with atherogenesis. For example, in a vulnerable plaque model using ApoE−/− mice, increased HO-1 expression positively associates with atherosclerotic plaque-containing metals, including cadmium and zinc. Overall, these findings provide a potential mechanism by which to account for the protective effects of metallothionein against eNOS-derived nitrosative stress in some vascular (and non-vascular) cell lines.

The relationship between vascular oxidant stress, HO-1, and NOS activation has also been studied. Durante et al demonstrated that cytokine-mediated increases in NO’ result in L-NAME-inhibitable upregulation of HO-1 protein expression levels in VSMC. Similar results have been observed in cultured vascular endothelial cells, in which treatment with the exogenous NO’ donor sodium nitroprusside, S-nitrosoglutathione, and 3-morpholinosynonimine upregulates HO-1. The functional effects of NO’-dependent HO-1 activation include sustained cell viability in the presence of pathological concentrations of oxidant stress (eg, H2O2: 250μmol/L). Moreover, by decreasing ROS levels, upregulation of HO-1 is, in turn, associated with restored eNOS activation despite conditions of increased oxidant stress. In a study of diabetic rats, low constitutive levels of eNOS expression in the aorta could be enhanced by pharmacologic stimulation of HO-1 using cobalt protoporphyrin. The redox-sensitive regulation of NO’-HO-1 signaling appears to depend, in part, on local thiol concentration levels. For example, SNAP- and hypoxia-dependent upregulation of HO-1 in vascular endothelial and smooth muscle cells, respectively, is attenuated in the presence of the glutathione precursor N-acetylcysteine, although the biological importance of this association is not fully resolved.

Conclusions

Discoversies over the previous decade have enhanced our understanding of the redox-dependent mechanisms involved in the regulation of eNOS-dependent signaling pathways in discrete subcellular compartments, both in the context of physiological oxidant signaling pathways and in disease states. The identification of subcellular targets of ROS, as well as of functional redox-sensitive cysteinyl thiols in eNOS and its signaling partners, has expanded the number of potential future treatment targets to modify cardiovascular diseases associated with impaired eNOS function. Because of the biologically diverse enzymatic sources of ROS functioning, both in normal tissues and in pathological oxidant stress, it is likely that therapies aiming to preserve eNOS signaling will require the identification of site-specific therapies rather than nonspecific antioxidant treatments alone. Efforts to identify druggable eNOS sites are ongoing; for example, systematic enrichment of ligands by exponential enrichment (SELEX) technology may represent a promising technology implemented to synthesize an eNOS site-selective aptamer against particular redox-sensitive cysteinyl thiol(s) in order to preserve eNOS function despite unfavorable local redox milieu. The success of these endeavors, however, is likely to hinge on a more comprehensive understanding of the underpinnings of the redox-sensitive mechanisms that regulate the eNOS-N0’ signaling axis both in normal vascular physiological signaling pathways and in disease states.

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Disclosures

None.

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