Interactions of Oxidants With Vascular Signaling Systems

Michael S. Wolin

Abstract—Individual reactive oxygen species (ROS) and oxidation products of NO interact with vascular signaling mechanisms in ways that appear to have fundamental roles in the control of vascular physiological and pathophysiological function. The activities of ROS-producing systems (including various NADPH and NADH oxidases, xanthine oxidase, and NO synthase) in endothelium and/or vascular smooth muscle are controlled by receptor activation, oxygen tension, metabolic processes, and physiological forces associated with blood pressure and flow. This review focuses on how the chemical properties and metabolic sensing interactions of individual ROS (including superoxide anion, hydrogen peroxide, and peroxynitrite) interact with cellular regulatory systems to produce vascular responses. These species appear to often function through producing selective alterations in individual heme or thiol redox–regulated systems (including guanylate cyclase, cyclooxygenase, mitochondrial electron transport, and tyrosine phosphatases) to initiate physiological responses through signaling pathways that control phospholipases, protein kinases, ion channels, contractile proteins, and gene expression. (Arterioscler Thromb Vasc Biol. 2000;20:1430-1442.)

Key Words: oxidants ■ redox ■ signaling, vascular

This review focuses on providing a logical rationale for the rather poorly understood mechanisms of how individual reactive oxygen species (ROS) interact with signaling systems of importance to vascular function. The interactions of oxygen and ROS with NO result in the generation of reactive nitrogen species (RNS), which possess additional oxidant signaling properties that need to be considered, because most individual ROS and RNS shown in the Figure have unique ways of interacting with cellular regulatory systems. There appear to be roles for oxidant signaling in acute physiological processes, such as the sensing of changes in PO2. As the levels of key species increase, they often participate in the activation of multiple types of pathophysiological responses, such as the attenuation of vasodilator mechanisms mediated through the stimulation of soluble guanylate cyclase (sGC) and the promotion of adhesion protein expression or vascular proliferative processes. When cellular antioxidant systems become overwhelmed, oxidant species then become activators of apoptotic or necrotic cellular injury. Thus, oxidant signaling mechanisms are of importance in vascular biological processes ranging from physiological responses to the alterations observed in vascular diseases.

Oxidant Species and Their Potential Interactions With Signaling Systems

Superoxide Anion

The production of ROS often begins with a 1-electron reduction of molecular oxygen to superoxide anion (O2•−) by various oxidases (Equation 1), which are discussed later in this article. Superoxide anion is a negatively charged free radical that undergoes rather selective chemical reactions with the components of biological systems. Although O2•− reacts with itself with a rate constant of 8×106 mol−1·L−1·s−1 to form H2O2 and O2 (Equation 2), superoxide dismutase (SOD) enzymes function to accelerate the removal of O2•− as a result of their rate constant of 2×107 mol−1·L−1·s−1 for the reaction with O2•−.

\[
(1) \quad \text{O}_2 + \text{electron} \rightarrow \text{O}_2^{•−}
\]

\[
(2) \quad \text{O}_2^{•−} + \text{O}_2^{•−} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2
\]

\[
(3) \quad \text{O}_2^{•−} + \text{NO} \rightarrow \text{ONOO}^{−}
\]

Vascular tissue contains a cytosolic copper-zinc form of SOD (CuZn-SOD), a mitochondrial manganese form of SOD (Mn-SOD), and an extracellular CuZn-SOD. One of the most important roles of SOD is the prevention of the reaction of O2•− with NO shown in Equation 3. It has been demonstrated that CuZn-SOD permits NO release from the endothelium and NO-mediated vascular smooth muscle (VSM) relaxation, whereas extracellular SOD appears to protect NO during its diffusion from endothelium to VSM. The activities of intracellular oxidases typically seen in vascular tissue should result in levels of O2•− in the nanomolar range in the absence of SOD, and the presence of SOD is likely to lower O2•− concentrations to the picomolar range. Picomolar levels of O2•− are not likely to have direct interactions with...
signaling mechanisms. However, these low levels of \( \text{O}_2^- \) can be a source, through reactions associated with Equation 2, of concentrations of \( \text{H}_2 \text{O}_2 \) in the high picomolar to low nanomolar range that interact with signaling systems. Because \( \text{O}_2^- \) reacts with NO with a rate constant of \( 7 \times 10^9 \text{mol}^{-1} \cdot \text{L} \cdot \text{s}^{-1} \), which is 3 times the rate of its reaction with SOD, when the levels of NO increase into the elevated nanomolar range and approach the local concentrations of SOD, NO is able to compete with SOD for the scavenging of \( \text{O}_2^- \).\(^7\)–\(^9\) This results in the production of peroxynitrite (\( \text{ONOO}^- \); see Equation 3) in amounts that can potentially interact with regulatory systems that are of biological significance.\(^7\)–\(^9\) As the levels of \( \text{O}_2^- \) increase, it readily interacts with iron-sulfur (Fe-S) centers at key cellular sites, including mitochondrial aconitase, causing prolonged inhibition of mitochondrial function.\(^10\) Superoxide also causes the release of iron, and the liberated iron can potentially participate in signaling processes through reduction to its ferrous (Fe\(^2+\)) form, which reacts with peroxide to form highly reactive “hydroxyl radical”–like species (“\( \cdot \text{OH} \)”), or it can promote oxidative stress–associated tissue injury.\(^11\) When nanomolar levels of \( \text{O}_2^- \) are formed in the extracellular environment in the absence of appreciable SOD activity, it readily attenuates the actions of NO\(^12\) and vasoactive catecholamines,\(^13\) including norepinephrine, epinephrine, and the drug isoproterenol. This occurs as a result of the direct chemical reactions between \( \text{O}_2^- \) and these vasoactive agents. Some of the potential mechanisms through which \( \text{O}_2^- \) and other ROS interact with vascular signaling systems are summarized in Table 1.

**H\(_2\)O\(_2\) and Peroxide Metabolism**

\( \text{H}_2 \text{O}_2 \) is a relatively stable species, with biological diffusion properties that are similar to \( \text{H}_2 \text{O} \). It is either derived from \( \text{O}_2^- \) through Equation 2, or it is directly produced by certain oxidases through a 2-electron reduction of \( \text{O}_2 \). A biologically significant source of other peroxides that interact with oxidant-related signaling systems are lipoxygenase enzymes, which typically metabolize arachidonic acid into hydroperoxyeicosatetraenoic acids species.\(^14\) It appears that the most sensitive and physiologically relevant mechanisms through oxidants are summarized in Table 1.

**TABLE 1. Sites of Interaction of Oxidant Species With Vascular Signaling Systems**

<table>
<thead>
<tr>
<th>Species</th>
<th>Site of Reaction</th>
<th>Signaling Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{O}_2^- )</td>
<td>NO</td>
<td>Inactivation of NO prevents sGC stimulation; ( \text{ONOO}^- ) generation.</td>
</tr>
<tr>
<td>Fe-S complexes</td>
<td></td>
<td>Inhibition of aconitase and mitochondrial respiration by releasing Fe, which forms ( \cdot \text{OH} ).</td>
</tr>
<tr>
<td>Catecholamines</td>
<td></td>
<td>Inactivation of epinephrine or norepinephrine inhibits interactions with adrenergic receptors.</td>
</tr>
<tr>
<td>H(_2)O(_2)</td>
<td>Catalase</td>
<td>Stimulates sGC.</td>
</tr>
<tr>
<td>GSH peroxidase</td>
<td></td>
<td>GSSG formation; see Table 2.</td>
</tr>
<tr>
<td>COX</td>
<td></td>
<td>Activation at low levels of peroxide.</td>
</tr>
<tr>
<td>( \text{PGI}_2 ) synthase</td>
<td></td>
<td>Inactivation at high levels of ROS and RNS.</td>
</tr>
<tr>
<td>( \cdot \text{OH} )</td>
<td>Thiol</td>
<td>Oxidation; see Table 2.</td>
</tr>
<tr>
<td>Lipids</td>
<td></td>
<td>Generation of vasoactive isoprostanes and lipid oxidation products.</td>
</tr>
<tr>
<td>NO</td>
<td>Heme of sGC</td>
<td>Stimulation of sGC.</td>
</tr>
<tr>
<td>Cytochrome oxidase</td>
<td></td>
<td>Reversible inhibition of respiration.</td>
</tr>
<tr>
<td>RNS</td>
<td>Thiol</td>
<td>See Table 2.</td>
</tr>
<tr>
<td>COX</td>
<td></td>
<td>Activation at low levels of ( \text{ONOO}^- ).</td>
</tr>
<tr>
<td>Tyrosine</td>
<td></td>
<td>Nitration-mediated inactivation of ( \text{PGI}_2 ) synthase.</td>
</tr>
<tr>
<td>Fe-S complexes</td>
<td></td>
<td>Inhibition of aconitase and mitochondrial respiration by releasing Fe, which forms ( \text{RSFe(NO)}_2 ) complexes.</td>
</tr>
</tbody>
</table>
which the various peroxide species interact with signaling systems are through processes linked to their metabolism by enzymes with peroxidase-like activities.

Catalase metabolizes H$_2$O$_2$, but not lipid peroxides, by initially reducing it to H$_2$O as a result of donating 2 electrons from its ferric heme, forming a highly oxidized heme intermediate called compound I (see Equation 4). The heme of compound I of catalase is then reduced back to its ferric form by oxidizing a second molecule of H$_2$O$_2$ to O$_2$ (see Equation 5). The rate constants for these 2 reactions result in the formation of compound I of catalase as H$_2$O$_2$ levels approach 1 nmol/L. As the levels of H$_2$O$_2$ increase, $\sim$40% of catalase exists as the compound I species. It has been demonstrated that the activity of sGC is activated by peroxide metabolism by catalase under conditions that are closely associated with the formation of the compound I species. Evidence has accumulated that H$_2$O$_2$ can produce vascular relaxation by stimulating sGC through studies that (1) examined the actions of agents that inhibit sGC stimulation by H$_2$O$_2$ (methylene blue and LY83583), (2) measured the association between changes in cGMP and vascular relaxation, and (3) characterized the actions of probes that inhibit H$_2$O$_2$ metabolism by catalase (3-amino-1,2,4-triazole, alcohols, ebselen, O$_2^-$, and NO). The properties of stimulation of sGC by H$_2$O$_2$ suggest that its expression is likely to be modulated by competing processes, including the efficiency of H$_2$O$_2$ metabolism by glutathione (GSH) peroxidase and by the levels of the physiological modulators of peroxide metabolism by catalase that inhibit sGC stimulation, including O$_2^-$, NO, and other tissue-derived electron donors for compound I of catalase, which remain to be identified.

\[ \text{Catalase} + H_2O_2 \rightarrow H_2O + \text{Compound I} \rightarrow \uparrow \text{cGMP} \]  
\[ \text{Compound I} + H_2O_2 \rightarrow O_2 + H_2O + \text{Catalase} \]

GSH peroxidase metabolizes H$_2$O$_2$ and other biological peroxides by reducing them with the use of electrons derived from the oxidation of GSH to its disulfide form (GSSG), as seen in Equation 6. The product of this pathway, GSSG, has the potential to regulate signaling systems (see Thiол Oxidation and Nitrosation) through promoting the S-thiolation (RSSG) of key protein thiols (RSH), as seen in Equation 7, because as the formation of GSSG increases, it appears to be used to form S-thiolated proteins. S-Thiolation can also promote disulfide formation [R(S-S)] with adjacent protein thiols when they are present.

\[ \text{ROOH} + 2 \text{GSH} \rightarrow \text{ROH} + \text{GSSG} \]  
\[ \text{GSSG} + \text{RSH} \rightarrow \text{GSH} + \text{RSSG} \]

Heme peroxidases present in tissues are additional enzymes with very high rates of reaction with peroxides. In mammalian systems, the metabolism of peroxide by these enzymes is often linked to the formation of signaling molecules, such as prostaglandins (PGs) by cyclooxygenase (COX) or the generation of additional ROS by myeloperoxidase. COX has an unusual mechanism of activation by peroxides, which seems to involve oxidizing its heme to a form that catalyzes the generation of PGs, and a peroxide produced by the COX reaction (PGG$_2$) appears to help sustain the production of PGH$_2$ by this enzyme. The availability of peroxide for metabolism by COX appears to be one of the most important mechanisms that control the biosynthesis of PGs by tissues. Certain biological lipid peroxides have much more efficient interactions with COX than does H$_2$O$_2$.

Peroxide-derived ROS, often described as species with hydroxyl radical (•OH)-like reactivity, may also be involved in signaling processes. Peroxides readily react with transition metals present in biological systems, such as iron, and this can result in the formation of species that are involved in signaling or tissue injury processes. The phagocytic cell myeloperoxidase metabolizes H$_2$O$_2$ to hypochlorous acid (HOCl), which can react with amines (eg, RNH$_2$) to form chloramines (eg, RNHCl). Although the myeloperoxidase-derived HOCI- and RNHCl-type species readily react with thiols and although chloramines appear to have significant biological activity, it is not yet known whether these actions are linked to the control of signaling mechanisms in a manner that is independent of the cytotoxic actions of these species. Myeloperoxidase is also able to form RNs with tyrosine-nitrating activity when both nitrite and H$_2$O$_2$ are present. The reaction of H$_2$O$_2$ with ferrous iron (Equation 8) results in the formation of •OH. Although the extremely high rate of reaction of •OH with most molecules in biological systems has resulted in much debate regarding its hypothesized role in signaling processes, there is substantial evidence suggesting that the reaction of peroxides with Fe$^{2+}$ bound to proteins or small molecular weight molecules can result in stabilized iron-bound species with •OH-like reactivity. These “•OH” species appear to have a much greater potential for catalyzing reactions that could be involved in signaling processes; eg, one could speculate that a reaction catalyzed by a specific “•OH” or an enzyme-bound species with •OH-like reactivity could be involved the selective oxidation of a key site on a protein involved in activation of a signaling process.

\[ H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + •OH \]

NO and RNS

The most significant interactions of NO with signaling systems involve its reaction with ferrous heme groups, certain other metal sites, and free radical species. Formation of RNS through the oxidation of NO or through its reaction with ROS appears to result in the production of species that have additional interactions with regulatory processes. The most potent actions of NO occur over the lower nanomolar concentration range, and they include binding to the heme groups of sGC and heme-copper complexes of cytochrome oxidase. The selective actions of NO through these proteins appear to originate from their rates of binding NO, which are in the range of $10^8$ mol · L$^{-1}$ · s$^{-1}$. This results in a stimulation of the production of cGMP (associated with processes including vasodilation and the inhibition of platelet aggregation and neutrophil adhesion to the endothelium) and a reversible inhibition of mitochondrial respiration (associated with an improvement of the efficiency of energy metabolism). At these low levels of NO, the reaction of NO with O$_2^-$ leads to an attenuation of the stimulation of sGC and a conversion of the reversible inhibition of respiration to what appears to be a prolonged, and perhaps irreversible, inhibition.
of respiration\textsuperscript{25} resulting from the release of iron from Fe-S sites other than cytochrome oxidase.\textsuperscript{26} Although O$_2^\cdot$ is known to cause an irreversible inhibition of respiration by damaging the Fe-S center of the Krebs’ cycle enzymeaconitase,\textsuperscript{10} it appears that the interaction with NO enhances the potency of O$_2^\cdot$ as an inhibitor of respiration.\textsuperscript{23} Thus, the interaction of NO with O$_2^\cdot$ seems to attenuate processes thought to be associated with the beneficial signaling actions of NO.

When NO concentrations increase into the range of the tissue levels of SOD, NO competes with SOD for the removal of O$_2^\cdot$ by forming ONOO$^-$. Nitrogen dioxide (NO$_2$) appears to be produced in significant amounts from ONOO$^-$. Significant amounts of NO$_2$ may also be formed from the H$_2$O$_2$-dependent myeloperoxidase reaction in the presence of low (RSNO) thiols.\textsuperscript{7–9,28,29} It appears that the modification by these actions of RNS could function to enhance oxidant sensitivity of NO and O$_2$ in hydrophilic environments.\textsuperscript{27} As the levels of ONOO$^-$ and RNS increase, these species have signaling effects on tissue function that are of potential physiological significance. The most potent effects of ONOO$^-$ and RNS appear to be thiol modifications that either affect the function of signaling systems or result in the production of tissue-derived donors of NO. Oxidized NO-derived species, including ONOO$^-$, NO$_2$, and N$_2$O$_3$, readily interact with GSH and other thiols in tissues to cause thiol oxidation or the formation of nitrate (RSNO$_2$) or nitrosated (RSNO) thiols.\textsuperscript{7–9,28,29} It appears that the modification by RNS of key thiols on proteins that possess regulatory functions can serve as a site of control of signaling (see Thiol Oxidation and Nitrosation). The most abundant of the RSNO$_2$ species are also likely to function as tissue storage forms and donors of NO. When iron is reductively released from tissue storage sites, such as ferritin, and from damaged Fe-S centers, it also has the potential to participate in the formation of NO donors through the generation of (RS)$_2$Fe(NO)$_2$ complexes.\textsuperscript{30} High levels of ONOO$^-$ also seem to form NO donors through the modification of alcohols and sugars to nitrate species, which release NO in the presence of thiols.\textsuperscript{31} Thus, the formation of RNS seems to be an important process in the interaction of oxidants with signaling systems.

RNS may also participate in cellular signaling processes through additional interactions with lipids (see Eicosanoids and PLs) and proteins. Certain key metabolic enzymes, including the Fe-S centers of the mitochondrial electron transport chain and aconitase, and thiols located on glyceraldehyde-3-phosphate dehydrogenase\textsuperscript{32} and creatine kinase\textsuperscript{33} appear to be readily modified by ONOO$^-$ and related RNS species. Some of these modifications could affect tissue function through altering the pathways and efficiency of energy metabolism in a manner that influences a signaling process, such as ATP-dependent potassium channels. The formation of ONOO$^-$ is associated with the inhibition of several key antioxidant systems, including catalase,\textsuperscript{9} GSH peroxidase,\textsuperscript{34} and the mitochondrial SOD or Mn-SOD,\textsuperscript{35} and these actions of RNS could function to enhance oxidant signaling or injury-associated processes. ONOO$^-$ and NO$\cdot$ cause the nitration of tyrosine groups on proteins. Cu/Zn-SOD\textsuperscript{35} and carbon dioxide\textsuperscript{36} appear to enhance the rates of certain ONOO$^-$-mediated tyrosine nitration reactions. A tyrosine group on PGI$_2$ synthase appears to be particularly sensitive to nitration by ONOO$^-$, resulting in inactivation of this enzyme.\textsuperscript{37} Tables 1 and 2 list many of the known metabolic and signaling systems that are potentially regulated by ONOO$^-$ and related RNS species.

### Thiol Oxidation and Nitrosation

Protein thiols seem to have markedly different sensitivities to modification by $S$-thiolation or thiol oxidation and nitrosation. Thus, the degree of oxidation of GSH to GSSG caused by the metabolism of peroxides, oxidants, or nitrosative stress may have a major influence on which systems are modulated by the redox status of GSH. It is likely that certain thiols will be very sensitive to chemical or enzymatic $S$-thiolation and that other protein thiols will only be modified at much higher levels of oxidant stress. As the levels of GSSG increase, its metabolism by GSH reductase will decrease the levels of NADPH, a cofactor that is potentially involved in the reduction of modified thiols through thioredoxin reductase and related systems\textsuperscript{45} (see Interactions Between Redox Control Mechanisms and Oxidant Signaling). Table 2 includes some of the potential linkages between proteins regulated by thiol redox and signaling mechanisms (discussed later in this article) that appear to be controlled by rather poorly understood thiol redox–related processes.

<table>
<thead>
<tr>
<th>Thiol-Containing System</th>
<th>Signaling or Metabolic Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH redox</td>
<td>GSSG formation by ROS and RNS regulates multiple systems by S-thiolation and disulfide formation, and it increases cellular levels of H$_2$O$_2$. GSH nitrosation/nitration generates NO donors.</td>
</tr>
<tr>
<td>Calcium-regulated potassium channels</td>
<td>ROS and RNS generally open these channels, causing hyperpolarization.</td>
</tr>
<tr>
<td>Tyrosine phosphatases</td>
<td>Inhibition by ROS and RNS increases tyrosine phosphorylation; roles in activating Ras/MAPKs, PLA$_2$, PLC, PLD, and PKC and autoactivation of tyrosine kinases, etc.</td>
</tr>
<tr>
<td>p21$^{^\text{CIP1}}$</td>
<td>Nitrosation activates p42/p44 MAPK.</td>
</tr>
<tr>
<td>Calcium channels</td>
<td>Thiol oxidation by ROS and/or RNS seems to inhibit microsomal calcium storage and release and plasma membrane calcium influx.</td>
</tr>
<tr>
<td>sGC</td>
<td>Potentially inhibited by thiol oxidation.</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>Inhibition by RNS impairs aspects of the energy metabolism.</td>
</tr>
<tr>
<td>Caspases</td>
<td>Most are activated by high levels of ROS associated with apoptosis; some are inhibited by NO.</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>Inhibition by ROS and RNS impairs glycolytic energy metabolism.</td>
</tr>
</tbody>
</table>
are likely to define the conditions under which a signaling system is selectively influenced by individual oxidant species. The actual rates of reactions of peroxides with thiols are generally rather slow. However, efficient modifications to oxidized thiols [RSSG, R(S-S), and RSO3] can potentially result from activation of the thiol group by interactions within the protein environment in which they are located and by peroxides being converted through metal chelation to more reactive “·OH.” In addition to the formation of the RSNOX reactive peroxides being converted through metal chelation to more reactive “·OH,” the protein environment in which they are located and by peroxides being converted through metal chelation to more reactive “·OH.” In addition to the formation of the RSNOx reactive peroxides being converted through metal chelation to more reactive “·OH.” In addition to the formation of the RSNOX reactive species (see NO and RNS), RNS can promote the generation of oxidized thiols [RSSG, R(S-S), and RSO3] and increased levels of GSSG. Disulfide [R(S-S) and RSSG] and sulfenic acid (RSOH) modifications are oxidized forms of thiols that can be readily reduced back to the original sulphydryl by thiols, other reducing agents, or protein redox systems. In contrast, further oxidation to sulfinic (RSO2) or sulfonic (RSO3) oxidation states is likely to result in an irreversible modification of the protein. Although nitrosated low molecular weight thiols (eg, GSNO) can also promote the formation of nitrosated or oxidized thiols on proteins through transfer of the NO group to protein thiols (transnitrosation), this process is more likely to occur in the extracellular environment, because it is inhibited by cellular levels of GSH. Thus, modifications of protein thiols to RSNOX, RSSG, R(S-S), and RSSG forms can potentially be part of signaling mechanisms controlled by individual ROS and RNS species because of the potential for reversibility and the fine-tuned control of these processes by the function of cellular redox systems. Certain tyrosine phosphatases, potassium channels, and the G protein p21ras appear to have a significant role in restoring the normal thiol redox status of proteins, and the function of this system appears to be dependent on the redox status of NADPH, a reducing cofactor used by this system. In addition to the influence of NADPH and GSH redox systems on H2O2-elicited stimulation of sGC, NADPH-linked oxidoreductase systems appear to prevent the expression of additional inhibitory mechanisms involving oxidation of the heme and/or thiols on sGC. Thus, the redox status of NADPH and GSH is likely to have a major influence on the expression of multiple oxidant-associated signaling mechanisms.

It is currently thought that the balance between the generation of NADPH by glyceraldehyde-3-phosphate dehydrogenase and the removal of cystosolic NADH by the functioning of mitochondrial shuttle systems and the lactate dehydrogenase reaction has a major influence on the redox status of cystosolic NADH, keeping it primarily in the form of NAD. Glucose metabolism by the sorbitol pathway could be an additional source of cystosolic NADH under hyperglycemic conditions. Recent studies have provided evidence that the levels of cystosolic NADH appear to control the activity of an O2·-producing NADH oxidase [see NAD(P)H Oxidases]. Within mitochondria, the redox status of NADH is thought to be determined by the balance between the availability of substrates for the Krebs’ cycle and the usage of NADH by the electron transport chain. The levels of intracellular calcium, P02, ADP, pH, NO, and other factors that influence mitochondrial membrane potential seem to function together to determine the redox status of mitochondrial NADH, whereas the redox status of components of the electron transport chain appear to control mitochondrial O2·- production (see Mitochondrial Systems). The redox status of mitochondrial NADH seems to be controlled by a balance between its generation from NADH by the transhydrogenase reaction and its use by systems such as the GSH reductase reaction. Although little is known about the importances of intra mitochondrial ROS signaling mechanisms, it is likely that NADPH and GSH redox would have a major influence over processes involving changes in mitochondrial thiol redox. The redox status and/or the availability of other antioxidant-associated systems becomes important when the levels of ROS and RNS result in the generation of additional free radicals or highly reactive oxidized metal species. Agents such as α-tocopherol, urate, various thiols, ascorbate, and perhaps other radical scavengers, including NO, appear to quench these reactive species in a manner that prevents their destructive actions. Thus, various biological redox systems have a major influence on controlling the production, levels, and function of signaling systems influenced by certain ROS and RNS. As the capacity of redox systems to control the levels of reactive species is exceeded, free radical–scavenging antioxidants function either to inac-

Interactions Between Redox Control Mechanisms and Oxidant Signaling

The various cell types in the vessel wall contain the redox systems that are typically seen in other cell types. However, the ability of a cell to maintain the normal redox status of a particular redox system is likely to be an important factor in the control of oxidant signaling systems. Although many aspects of the function of redox systems remain poorly understood, certain facets of the redox status of cystosolic NADP(H), NAD(H), and GSH suggest that the redox status of these systems is a major contributor to the processes that influence the expression of oxidant-linked signaling mechanisms. It is currently thought that the pentose phosphate pathway of glucose metabolism is a major contributor to maintaining the majority of NADP(H) in its reduced form. NADPH-dependent GSH reductases are thought to be the major enzyme systems that maintain GSH in its reduced form. As GSH oxidizes to GSSG during the metabolism of peroxide or as a result of its interaction with reactive species, the increased level of GSSG appears to result in a substantial increase in proteins that have been S-thiolated with GSH. Thioredoxin reductase and related enzymes are thought to have a significant role in restoring the normal thiol redox status of proteins, and the function of this system appears to be dependent on the redox status of NADPH, a reducing cofactor used by this system. In addition to the influence of NADPH and GSH redox systems on H2O2-elicited stimulation of sGC, NADPH-linked oxidoreductase systems appear to prevent the expression of additional inhibitory mechanisms involving oxidation of the heme and/or thiols on sGC. Thus, the redox status of NADPH and GSH is likely to have a major influence on the expression of multiple oxidant-associated signaling mechanisms.
ticate these reactive species or to scavenge the free radicals that they generate.

**What Controls the Production of ROS and Their Actions on Signaling Systems?**
This section discusses the processes that control ROS production by many of the better understood oxidases (see Table 3) as well as aspects of the function of metabolizing systems for these species that influence the interaction of ROS and RNS with signaling systems. The production and metabolism of ROS are highly compartmentalized. For example, the cytosol, mitochondria, peroxisomes, and extracellular region appear to function as environments whose production and metabolism of ROS generally appear to be rather independent of each other.15

**Control of the Production of Oxidant Species**
It is important to keep in mind that each active source of production of ROS within a compartment contributes to the local levels of each species and that transport of individual ROS across compartments is often possible when the capacity of the scavenging systems is exceeded.

**NAD(P)H Oxidases**
There are several known NAD(P)H oxidases. Phagocytic cells have a membrane-bound flavohemoprotein containing NADPH oxidise with cytochrome b558, which produces minimal amounts of O$_2$- until it is stimulated by signaling processes associated with cellular activation. The membrane-bound gp91phox and p21phox subunits are thought to contain the flavoprotein and heme sites, and p47$^{phox}$, p67$^{phox}$, p40$^{phox}$, and the G protein rac-2 appear to bind the membrane-bound subunits on cellular activation.48 This binding is associated with the generation of a highly active NADPH oxidase capable of producing large amounts of O$_2$- on the external surface of the plasma membrane. Although many of the subunits of this NADPH oxidase appear to be present in vascular endothelium and VSM,49–58 it is not yet known whether these subunits are regulated and function in a manner similar to the phagocytic cell oxidase. Thus, NADPH oxidase is an important source of ROS in segments of the circulation that are exposed to activated inflammatory cells.

A somewhat similar NAD(P)H oxidase was identified in endothelium49 and VSM.50,51 This oxidase appears to have a basal NAD(P)H-dependent O$_2$-generating activity in the absence of cellular activation, and certain stimuli, such as angiotensin II,51 tumor necrosis factor-α,52 thrombin,53 and lactosylceramide,54 appear to stimulate the activity and/or expression of this protein. Although there is evidence that endothelium55 and VSM56–58 contain many of the components analogous to the p21, p47, p67, and gp91 subunits of the phagocytic oxidase, the role of most of these proteins in the regulation of the vascular NAD(P)H oxidase requires further study. It has recently been reported that the vascular NAD(P)H oxidase has a mox-1 (or p65mox) subunit that (on the basis of sequence homology) appears to be analogous to the p91$^{phox}$ subunit of the phagocytic oxidase.57 In human aortic VSM cells, activation of NAD(P)H oxidase by thrombin is associated with increased expression and membrane binding of p47$^{phox}$ and rac-2.53 Angiotensin II was also observed to cause membrane binding of p47$^{phox}$ in bovine pulmonary arteries under conditions that increased NADH oxidase activity.56 Expression of the p21$^{phox}$ and mox-1 subunits is observed to be associated with O$_2$- generation and growth in VSM cells.51,57 Studies in bovine pulmonary and coronary arterial smooth muscle and coronary artery endothelial cells have provided evidence that a potentially important control mechanism for NADH oxidase activity appears to be the availability of cytosolic NADH (see Interactions Between Redox Control Mechanisms and Oxidant Signaling).17,46,49,50,59 The rate of O$_2$- production by the bovine VSM NADH oxidase seems to be dependent on the concentration of O$_2$ in a manner that permits this oxidase to function as a physiologial O$_2$ sensor.17,50 Thus, NAD(P)H oxidases may be a key source of ROS that participate in vascular oxidant–related signaling mechanisms under physiological and pathophysiologic conditions.

There are additional NAD(P)H oxidases that may contribute to vascular ROS signaling. Cytochrome P-450 is known to be a source of O$_2$- production through its NADPH oxidase activity. The primary cytochrome P-450—type enzyme observed to be a significant source of ROS in the vessel wall is the endothelial form of NO synthase (NOS). The various forms of NOS have NADPH oxidase activity. It has been demonstrated that this activity is markedly enhanced in endothelium as a result of a deficiency of its cofactor tetrahydrobiopterin50 and also as a result of exposure to atherogenic levels of LDL.41 COX is an additional source of O$_2$- production during the synthesizing of PGs or metabolizing of peroxides because of its ability to co-oxidize substances such as NAD(P)H.62 COX has been observed to be a significant source of O$_2$- in the cerebral circulation.63 Thus, various pathophysiologic conditions seem to be associated

<table>
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<tr>
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<th>Processes That Control Oxidant Production</th>
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with specific NAD(P)H oxidases becoming significant vascular sources of ROS.

**Xanthine Oxidase**
The xanthine dehydrogenase activity present in vascular endothelium is readily converted into xanthine oxidase by processes including thiol oxidation and/or proteolysis. Xanthine oxidase metabolizes hypoxanthine, xanthine, and NADH to form O$_2^-$ and H$_2$O$_2$. Ischemia and hypoxia are conditions that promote the accumulation of these substrates for ROS production and the increases in xanthine oxidase activity. Xanthine oxidase appears to be an important source of ROS production in ischemia/reperfusion and hypercholesterolemia. Thus, xanthine oxidase has the potential to be an important source of ROS production under certain pathophysiological conditions.

**Mitochondrial Systems**
The significance of the production of ROS by mitochondria in vascular signaling is rather poorly understood. Mitochondria are thought to produce O$_2^-$ from the semiquinone form of coenzyme Q and a reduced component of NADH dehydrogenase. It appears that inhibition of mitochondrial respiration by NO can result in increases in mitochondrial ROS production. On the basis of the actions of rotenone, it has been suggested that mitochondrial-derived ROS influence hypoxic pulmonary vasoconstriction (see Roles for Oxidant Signaling and Vascular O$_2$-Sensing Mechanisms). Mitochondrially derived ROS could also be important contributors to the expression of apoptosis.

**Antioxidant and Metabolic Control of ROS Levels and Actions**
The influence of metabolizing or scavenging systems on the levels individual ROS (and RNS) may be a key aspect that determines the expression of signaling processes regulated by these species. This article has already considered the roles of SOD, catalase, and GSH peroxidase in controlling the levels of O$_2^-$ and peroxides, the interaction of O$_2^-$ with NO, and the ways through which peroxide metabolizing enzymes interact with sGC, thiol redox–linked signaling, and PG biosynthesis. The expression of these ROS-scavenging systems appears to be highly regulated by environmental factors, such as previous exposure to oxidant stress. In addition, as considered in Interactions Between Redox Control Mechanisms and Oxidant Signaling, certain key cellular redox systems have a major influence on the function of signaling processes activated by ROS.

**Relationships Between Cellular Control Mechanisms Regulated by ROS and Interactions of ROS With Signaling Systems**
This section focuses on discussing the manner through which ROS and RNS interact with some of the vascular control systems that appear to be regulated by redox-associated processes.

**Ion Transport Systems**
Reports that elevated levels of H$_2$O$_2$ cause calcium-dependent release of NO from the endothelium and potassium channel–dependent relaxation of VSM stem from observations demonstrating the potential importance of oxidant regulation of ion transport mechanisms. Although the actual links between ROS or RNS and the function of cellular ion transport systems are generally not well understood, evidence exists for the potential importance of several processes. The mechanisms that control the uptake and/or release of sarcoplasmic reticulum or microsomal calcium in VSM and endothelium appear to be rather sensitive to oxidants. The oxidation of thiols that control the activities of these ion transport systems has often been considered to be a mechanism of regulation. Plasma membrane potassium channels in VSM that control a hyperpolarization-elicited relaxation appear to be opened by mechanisms associated with thiol oxidation by ROS or thiol modification by RNS. Calcium-regulated potassium channels appear to mediate the vasodilation to H$_2$O$_2$ in the rat cerebral microcirculation. Interestingly, in the cat cerebral microcirculation, O$_2^-$ was reported to produce dilation by calcium-dependent potassium channels, whereas H$_2$O$_2$ and ONOO$^-$ appeared to cause dilation through the opening of ATP-dependent potassium channels. Other signaling systems that are regulated by oxidants, such as cGMP-dependent processes, also control the function of ion channels. A recent study of the mechanism of relaxation of endothelium-removed bovine coronary arteries to diamide, an oxidant of GSH and adjacent protein thiols, has provided evidence for a mechanism involving the inhibition of plasma membrane calcium influx. In addition, the mechanism of relaxation of these arteries to diamide does not appear to involve modulation of sGC, potassium channels, O$_2^-$ dependent processes, or signaling systems associated with the release of intracellular calcium or contraction elicited by protein kinase C (PKC). Thus, vascular ion channels are potentially controlled by multiple redox-linked mechanisms, and this is likely to be responsible for the diversity of observations that have been made.

**Protein Phosphorylation**
The function of multiple components of protein phosphorylation systems has been shown to be altered by ROS and RNS. It is important to emphasize that a specific phosphorylation could be controlled by interactions of ROS or RNS with an independent signaling system (eg, calcium, cGMP, or diacylglycerol) that influences the function of the protein kinase catalyzing the phosphorylation or the activity of a phosphatase that removes the phosphate group. For example, signaling through cGMP-dependent protein kinases is likely to be highly regulated by the status of ROS, RNS, and redox systems because of their influence on the activity of sGC. Tyrosine-specific protein phosphatases have been demonstrated to have an essential thiol at their catalytic site, and modification of this thiol by either ROS or RNS is a potentially important biological mechanism that inhibits these proteins. The stimulation of tyrosine phosphorylation by H$_2$O$_2$ has been reported to be a mechanism of activating most forms of PKC, and enzyme activation seems to be independent of diacylglycerol generation. Evidence is emerging that redox processes markedly influence the balance of the activities between the various mitogen-activated protein kinase (MAPK) systems that appear to regulate vascular force generation, proliferation, and adaptive responses to injury. The function of the extracellular signal–regulated kinases, including p42/p44 MAPK, stress-activated or c-Jun...
N-terminal kinase, and the p38 MAPK–associated pathways, all seem to be significantly influenced by redox processes. It has been recently reported that the angiotensin II receptor and H₂O₂ can also activate protein kinase B by a phosphatidylinositol 3-kinase–dependent mechanism. Some of the sites with which ROS and RNS potentially interact in the control of MAPK systems appear to be tyrosine phosphatases, the small molecular weight G protein p21ras, PKC, and sGC. The impact of inhibition of tyrosine phosphatases by thiol redox processes would be to cause an apparent enhancement of upstream protein kinase–linked signaling systems that are partially activated under physiological or pathophysiological conditions. In addition, there is evidence that oxidant mechanisms may stimulate increases in the autophosphorylation of receptor-linked tyrosine kinases that activate the MAPK pathways, and H₂O₂ has been observed to stimulate tyrosine phosphorylation of the epidermal growth factor receptor in VSM cells, whereas nitrosation of a thiol on p21ras stimulates the activation of p42/p44 MAPK. One area that is very poorly understood is how individual ROS and RNS influence the balance between the activities of the MAPK pathways. For example, the simultaneous activation of p21ras and PKC are potentially key processes that stimulate the p42/p44 MAPK pathway via activation of Raf in VSM. A phosphorylation mediated by cGMP-dependent protein kinase has also been demonstrated to activate the p42/p44 MAPK pathway in VSM. Interestingly, the p42/p44 MAPK pathway may mediate receptor-stimulated calcium-independent contractile response in VSM through the phosphorylation of caldesmon. This field seems to be extremely important because of its apparent role in influencing adaptive responses of the vessel wall to altered physiological states and injury. Thus, ROS and RNS have multiple ways of interacting with processes that control the expression of responses linked to the various protein phosphorylation systems that are normally part of receptor-regulated signaling systems. Because the inhibition of tyrosine phosphatases is often a key process through which oxidants interact with protein phosphorylation–linked signaling systems, the basal activities of these signaling systems under physiological conditions and the extent to which individual ROS and RNS influence these signaling systems are likely to be key factors in determining the observed vascular responses.

Eicosanoids and PLs
H₂O₂ has been reported to stimulate multiple forms of vascular phospholipases (PLs), including PLA₂, PLC, and PLD. and ROS and RNS modulate the activity of arachidonic acid–metabolizing enzymes and directly modify lipids to species that are vasodilatory. The signaling pathways through which ROS control the activity of PLs are rather poorly understood. Cytosolic PLA₂ activity appears to be stimulated by H₂O₂ through a tyrosine kinase–dependent pathway, potentially involving the phosphorylation of PLA₂ by the activation of MAPK and PKC. There is evidence that tyrosine phosphorylation also contributes to the peroxide-mediated stimulation of PLC and PLD, and PKC is potentially an important participant in the activation of PLD. Activities of the lipoxygenase and the previously discussed COX enzymes are stimulated by low levels of peroxides, and peroxynitrite appears to stimulate COX in a manner similar to that of peroxides. Elevated levels of peroxides appear to inactivate COX and PGI₂ synthase, and PGI₂ synthase is also inactivated by pathophysiological levels of ONOO⁻. Thus, oxidants are potent stimuli in the activation of PLs and the generation of certain eicosanoids. Oxidative or nitrosative stress appears to alter the metabolites formed in a manner that reduces the generally beneficial effects of PGI₂ while increasing the levels of metabolites such as PGH₂, which may contribute to pathophysiological responses, including thrombosis and vasoconstriction.

Products derived from direct chemical reactions between ROS or RNS and lipids may also generate lipid-derived species with biological activity. As a result of its antioxidant activity, NO can add to lipid radicals (L•, LO•, and LOO•) to form lipid NO-containing species by the reactions shown in Equation 17. Although little is known about the biological activities of these NO-containing lipids, they are likely to function as tissue storage forms of NO. One of the products of the reaction of ONOO⁻ with arachidonic acid appears to be α-hydroxy nitro–containing eicosanoids [R-C(OH)-C(NO₂₋₋R)], which spontaneously release NO and cause vascular relaxation. Several of the metabolites considered to be indicators of lipid peroxidation, including isoprostanes and 4-hydroxy-2-nonenal, have been shown to have biological activities. Chemical interactions between lipids and ROS or RNS appear to generate species that potentially have signaling actions that influence vascular function, yet the actual roles of these species remain to be defined. l-NO, LOO•+NO→LNO, LONO, LOONO

Gene Expression
Redox processes that are influenced by ROS and RNS appear to have a major role in modulating gene and protein expression through the regulation of transcription factors (eg, nuclear factor-κB and activator protein-1) and many additional mechanisms, which have been considered in a recent review. Although this field is beyond the scope of the present article, many important aspects of ROS signaling can potentially be markedly altered through changes in gene expression. For example, redox signaling regulates the expression of (1) adhesion proteins that control inflammatory cell recruitment, (2) antioxidant enzymes that control all aspects of ROS interactions with signaling systems, (3) NOS, (4) receptors, and perhaps (5) many respiratory adaptations to hypoxic environments. Thus, the modulation of protein and gene expression by ROS and RNS also appears to be an important aspect of the behavior of the signaling systems these species regulate.

Vascular Processes Regulated by ROS Signaling Systems
There is rapidly growing literature on the effects or role of ROS and RNS on signaling systems. This section considers how the fundamental interactions of ROS with signaling systems are potentially linked to some of the better understood vascular biological processes that are known to be regulated by ROS.
Roles for Oxidant Signaling and Vascular O₂-Sensing Mechanisms

One of the first vascular responses suggested to be mediated through oxidant signaling mechanisms was the contractile response of the pulmonary vasculature to hypoxia. At least 2 hypotheses involving ROS are being actively considered for the mechanism of this response. Both mechanisms share the concept that hypoxia is removing a dilator mechanism controlled by ROS. Weir and colleagues have suggested that mitochondrial and cytosolic oxidases in pulmonary arterial smooth muscle are controlling the redox status of key thiols on plasma membrane voltage-regulated potassium channels through diffusible cytosolic redox cofactors. Normoxia maintains the channels in an open state, causing decreased force generation through hyperpolarization by oxidizing thiols on the channels. Our group has hypothesized that H₂O₂ derived from O₂⁻ produced by an NADH oxidase, whose activity is controlled by cytosolic NAD(H) redox and P₅₀, decreases force generation under normoxia. This is a result of the stimulation of cGMP production by sGC being activated by the metabolism of H₂O₂ by catalase. PGs are often observed to mediate vascular responses caused by changes in P₅₀. Stimulation of the production of either dilator or constric- tor PGs by increases in H₂O₂ under conditions such as posthypoxic reoxygenation has been suggested to mediate some of the responses that are observed. After inhibition of the PG-mediated responses, these vascular segments show H₂O₂-elicited responses that appear to be mediated through the stimulation of sGC. Gestational diabetes appears to enhance the H₂O₂-elicited PG-mediated contractile responses by a mechanism that seems to involve an attenuated expression of the simultaneous H₂O₂-elicited stimulation of sGC through a process that may involve the inhibition of catalase activity by increased levels of NO production. Because vascular oxidases, such as NADH oxidase, show changes of rates of ROS production at physiologically relevant O₂ tensions, these systems are likely to function as vascular O₂ sensors that activate signaling mechanisms involved in PG-elicited changes in force and perhaps environmental adaptations involving gene expression and proliferation.

Potential Roles for Oxidant Signaling in Vascular Responses to Receptor Agonists, Pressure, and Flow

Some of the responses to other physiological processes, such as receptor activation, pressure, and flow, may involve ROS-mediated signaling. It was initially observed in the cerebral circulation that activation of bradykinin receptors and acute elevations in pressure stimulated an increased production of extracellular free radicals, such as O₂⁻. COX activity in the endothelium appeared to be the primary source of these radicals. Subsequent studies identified multiple roles for oxidant signaling in vascular regulatory processes. This section emphasizes examples of roles for intracellular oxidant signaling mechanisms that appear to link physiological stimuli to some of the alterations that seem to occur in vascular function.

Receptors and Vascular Proliferative Signaling

The growth-promoting agent angiotensin II was observed to increase the activity of NAD(P)H oxidase activity in VSM. Further studies on the oxidant-mediated growth-promoting actions of angiotensin II in VSM cells have identified what appear to be essential roles for H₂O₂, the activation of protein kinase B, PKC, and the p42/p44 MAPK systems. Other vascular growth-promoting agents, including serotonin, have been demonstrated to stimulate the formation of oxidants and activate phosphorylation of p42/p44 MAP kinase. The potential mechanisms through which ROS interact with these protein kinase–linked pathways is discussed in Protein Phosphorylation.

Flow, Shear, and Stretch as an Initial Stimulus for Endothelial Oxidant Signaling and Subsequent Alterations in NO Regulation

Flow was also shown to be a stimulus for the production of free radicals from the endothelium of intact vascular tissue. Studies on the effects of flow on cultured human umbilical vein endothelium have identified the initial activation of NADH oxidase activity and a subsequent increase in CuZn-SOD expression by exposure to steady laminar shear. In this system, oscillatory stretching or shear stress caused a sustained activation of pro-oxidant processes associated with redox-sensitive gene expression. In a bovine aortic endothelium cyclic stretching strain-type model, it was observed (with the use of dominant-positive and -negative mutant cell lines) that this stimulus activated the p21ras-Raf-p42/p44 MAPK pathway through the production of H₂O₂. When subjected to laminar shear stress, cultured bovine aortic endothelium also shows evidence of increased oxidant signaling involving activation of the c-Jun N-terminal kinase pathway by ONOO⁻, and this may occur as a result of the upregulation of NOS. Although shear stress appears to increase endothelial cell NOS expression through mechanisms that seem to be independent of the actions of ROS, H₂O₂ has been reported to increase the expression of NOS by processes associated with increased transcription and mRNA stability. Although the actual mechanisms through which the forces associated with changes in blood flow influence the production of oxidant species is not known, alterations in signaling mechanisms activated by ROS and RNS could participate in the adaptation of vascular responses through changes in the expression of proteins that modulate redox signaling, such as increasing the expression of SOD. Thus, the shear forces caused by blood flow can influence endothelial signaling mechanisms and gene expression through changes in ROS and RNS production. These processes may participate in adaptations of vascular function to stimuli such as exercise.

Pressure or Wall Stress as a Stimulus for Oxidant Signaling

The initial effects of pressure on vascular tissue appear to be the release of O₂⁻ from endothelium-derived sources. For example, exposure of isolated rat skeletal muscle arterioles to elevated luminal pressure was observed to cause an O₂⁻-mediated attenuation of endothelium-dependent relaxation. An endothelium-independent contractile response of isolated cat cerebral arteries to increases in flow has recently been reported to be altered by probes that suggest a role for cell surface integrins, O₂⁻, and tyrosine kinases in the mechanism of this response. Thus, oxidant signaling is potentially
important in the acute responses of vascular tissue to increases in pressure or wall stress.

**Oxidant Signaling and Pathophysiological Aspects of Endothelial-Vascular Function**

There appear to be multiple ways through which pathophysiological conditions in the vasculature can promote the activation of oxidant signaling mechanisms. Some of the better understood roles for these mechanisms in vascular pathophysiology are briefly highlighted here.

**Origins of Alterations in Endothelial Oxidant-NO Signaling**

Some of the stimuli for alterations in oxidant-NO signaling were considered in Roles for Oxidant Signaling and Vascular O$_2$-Sensing Mechanisms and Potential Roles for Oxidant Signaling in Vascular Responses to Receptor Agonists, Pressure, and Flow. The vascular endothelium appears to have multiple potential sources of ROS production, including xanthine oxidase, NAD(P)H oxidases (including NOS, other cytochrome P-450s, and p21$^{phox}$-cytochrome $b_{55}$-containing oxidases), COX, and mitochondria. The most dominant initial effect of increased ROS production by endothelium appears to be the attenuating action of O$_2$ in NO signaling. Enhancement of this interaction seems to occur in multiple vascular diseases as a result of increased O$_2^-$ production through different systems, including xanthine oxidase, NAD(P)H oxidase, and NOS. Because peroxides have been observed to stimulate NOS activity by elevating endothelial cell calcium and NOS expression, as the production of oxidants and NO increases in the endothelium or other compartments in the normal or diseased vessel wall, ONOO$^-$ formation is likely to occur and have a major signaling role. In the absence of adequate levels of NO, the pathophysiological effects of ROS are likely to dominate the signaling and oxidative stress responses that are observed.

The alterations in endothelial NO signaling caused by increased O$_2^-$ production contribute to important processes, such as the promotion of vasoconstriction or vasospasm, attenuation of the inhibition of platelet aggregation, and promotion of neutrophil adhesion. Elevated levels of NO will generally reverse these actions of O$_2^-$. Although increases in NO also potentially influence regulatory systems in the vessel wall through the formation of RNS, the modulation of thiol redox, the generation of NO donors, and the inhibition of tissue catalase activity and mitochondrial function, the role of these NO-elicited processes in pathophysiological situations is not well understood. The effects of a simultaneous elevation of NO and O$_2^-$ will probably be dominated by the actions of ONOO$^-$, which will change as tissue antioxidant systems such as GSH become stressed and antioxidant enzymes become inactivated. Because exposure of coronary arteries to hypoxia/reoxygenation results in an ONOO$^-$-mediated inactivation of PGI$_2$ synthase and increased formation of unmethylated PGH$_1$, this may be one of the most prominent pathophysiological signal–like effects of ONOO$^-$ in vascular diseases.

**Pathophysiological Consequences of Alterations in Endothelial–Vascular Oxidant NO Signaling**

One of the first pathophysiological conditions observed to activate the production of increased levels of endothelium-derived ROS and ROS-mediated signaling responses was ischemia/reperfusion. In general, ischemia/reperfusion appears to cause an acute increase in the production of ROS and RNS by endothelium, associated with an attenuation of NO signaling and activation of an inflammatory response that often promotes a progression of the tissue injury caused by ischemia. Interestingly, the signaling and oxidant-scavenging activities of increased levels of NO appear to attenuate many aspects of acute injury and the subsequent inflammatory response caused by ischemia/reperfusion.

**Concluding Remarks**

Oxidant signaling mechanisms are now being recognized for their extremely important role in the control of vascular physiological and pathophysiological function. A large number of rather poorly understood signaling mechanisms seem to exist, and they appear to be controlled by the levels of each of the species present, by the tissue compartment in which the ROS are being formed, and by the degree of influence the species have on antioxidant defense mechanisms and key cellular redox systems. Most dietary antioxidants appear to have only modest physiological effects because they seem to protect against the consequences of “OH” and ONOO$^-$ generation, such as lipid peroxidation, whereas the cellular concentrations of these antioxidants are likely to have only...
minimal effects on signaling mediated by O$_2^-$, H$_2$O$_2$, NO, and thiol redox processes. Pharmacological agents that modulate the levels of specific reactive intermediates or that modulate enzymes involved in oxidant signaling pathways may have greater potential for therapeutic effects. Although a fine-tuned balance between the activation and adaptation of multiple mechanisms controlled by oxidative or nitrosative signaling probably occurs in most chronic vascular diseases, these adapted systems are more likely to respond in an abnormal manner under stressful conditions.

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Interactions of Oxidants With Vascular Signaling Systems
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