NADPH Oxidases: Functions and Pathologies in the Vasculature

Bernard Lassègue, Kathy K. Griendling

Abstract—Reactive oxygen species are ubiquitous signaling molecules in biological systems. Four members of the NADPH oxidase (Nox) enzyme family are important sources of reactive oxygen species in the vasculature: Nox1, Nox2, Nox4, and Nox5. Signaling cascades triggered by stresses, hormones, vasoactive agents, and cytokines control the expression and activity of these enzymes and of their regulatory subunits, among which p22phox, p47phox, Noxa1, and p67phox are present in blood vessels. Vascular Nox enzymes are also regulated by Rac, ClC-3, Poldip2, and protein disulfide isomerase. Multiple Nox subtypes, simultaneously present in different subcellular compartments, produce specific amounts of superoxide, some of which is rapidly converted to hydrogen peroxide. The identity and location of these reactive oxygen species, and of the enzymes that degrade them, determine their downstream signaling pathways. Nox enzymes participate in a broad array of cellular functions, including differentiation, fibrosis, growth, proliferation, apoptosis, cytoskeletal regulation, migration, and contraction. They are involved in vascular pathologies such as hypertension, restenosis, inflammation, atherosclerosis, and diabetes. As our understanding of the regulation of these oxidases progresses, so will our ability to alter their functions and associated pathologies. (Arterioscler Thromb Vasc Biol. 2010;30:653-661.)

Key Words: atherosclerosis ■ blood vessels ■ hypertension ■ NADPH oxidase ■ reactive oxygen species

Our knowledge of the signaling role of reactive oxygen species (ROS) in vascular physiology and pathophysiology has expanded tremendously in the past 15 years. NADPH oxidases (Nox) have emerged as major sources of ROS in the vasculature, multiple Nox subtypes have been cloned and analyzed structurally and functionally, and the relationship of Nox enzymes to signaling pathways, cellular function, and vascular disease has begun to be investigated.

The Nox family consists of 7 catalytic homologues, 4 of which (Nox1, Nox2, Nox4, and Nox5) are found in the vasculature. These enzymes transfer electrons from NADPH to molecular oxygen, thus producing superoxide (O$_2^-$). Because O$_2^-$ does not readily cross membranes and is short-lived, its effect is mostly local. Depending on Nox subcellular location, O$_2^-$ is released either inside organelles or extracellularly, with corresponding internal signaling or paracrine effects. Superoxide dismutase rapidly converts O$_2^-$ to longer-lasting and membrane-diffusible H$_2$O$_2$, thus modifying the signal and expanding its range of action. Whereas some effects of Nox enzymes, such as inactivation of nitric oxide (NO) in blood pressure regulation, are mediated directly by O$_2^-$, many are instead attributable to protein modification by H$_2$O$_2$, including growth-related signal transduction in vascular smooth muscle cells (VSMC).

In this brief review, we discuss our current understanding of vascular NADPH oxidases, especially their roles in physiology and disease. We first describe each oxidase separately, before presenting an overview of their interactions and connections with other systems.

Nox1
Nox1 is expressed in endothelium, smooth muscle, and adventitial fibroblasts, at the plasma membrane, caveolae, and endosomes. Interestingly, in VSMC, Nox1 is complexed with the novel activator Noxa1 and the phagocytic organizer p47phox (Figure 1). Unlike its homologue Noxo1, p47phox requires activation by phosphorylation, allowing for regulation of enzymatic activity. The identity of Nox1 regulatory subunits in other vascular cells is not known. Nox1 also closely associates with protein disulfide isomerase, a chaperone essential to its activity.

Other proteins essential to Nox1 activity do not necessarily associate with it. Because Nox enzymes carry electrons across membranes, they require charge compensation. Agonist-induced Nox1 activity in VSMC endosomes is blocked by deletion of the chloride/proton antiporter CIC-3 and rescued by transfection of intact channels. By exchanging protons for chloride anions, the antiporter prevents...
oxidase-induced cytosol acidification and accumulation of endosomal negative charges (Figure 1). Targeting anion channels could be used to inhibit oxidases, but their tissue and Nox subtype specificity remain to be investigated.

Nox1 is most highly expressed in intestinal epithelium, because of transcriptional upregulation of its promoter by cytokines involved in host defense. In VSMC, Nox1 is induced by growth factors and vasoactive agents (Table). This results from preferential activation of another promoter, located further upstream in the gene, in VSMC stimulated to grow by injury or angiotensin II (AngII). The resulting longer transcript produces a protein with a slightly longer N-terminus, whose activity is identical to epithelial Nox1. This promoter is responsible for Nox1 upregulation by bone morphogenetic protein-4, a Nox1-dependent endothelial cell (EC) agonist. More directly, Nox1 overexpression increases serum-induced proliferation in VSMC treated with platelet-derived growth factor (PDGF) or prostaglandin F2α via a signaling cascade including protein kinase C-δ (PKCδ), epidermal growth factor (EGFR) transactivation, and either PI3K, ATF-1, and MEF2B or ERK1/2 and JunB. Binding sites for transcription factors MEF2B and JunB have been identified.

Agonists also acutely activate Nox1 and its regulatory subunits by stimulating upstream signaling cascades, including phospholipases C and D, PKC, Src, and PI3K. These pathways are required for activation of p47phox and Rac. ROS produced by Nox1 then regulate downstream signaling pathways and essential cellular functions (Table), as discussed in greater detail elsewhere.

The role of Nox1 in cardiovascular disease has been studied mainly in hypertension. Vascular or kidney Nox1 is upregulated in experimental models, such as renin transgenics, implying that other mechanisms control chronic hypertension. Because growth factors stimulate ROS production in VSMC, the role of Nox1 in proliferative vascular disease was investigated. Nox1 is activated or upregulated in VSMC treated with AngII, low-density lipoprotein, or advanced glycation end products. Nox1 overexpression increases serum-induced proliferation and PDGF-induced migration in VSMC. Conversely, Nox1 deletion inhibits VSMC proliferation induced by serum, thyroid hormone, or PDGF, as well as PDGF or basic fibroblast growth factor-induced migration, which is rescued by Nox1 transfection. Moreover, Nox1 is activated or upregulated in vessels from diabetic animals. Remarkably, not only is injury-induced intimal hyperplasia accompanied by Nox1 upregulation but also it is inhibited by Nox1 deletion. Thus, Nox1 can clearly mediate abnormal vascular growth and possibly atherosclerosis.

Conversely, Nox1 deletion improves AngII-induced hypertension, but not ROS-independent norepinephrine-induced hypertension. Unexpectedly, Nox1 deletion prevents AT1R cell surface expression. Whatever the mechanisms, these data implicate Nox1 in AngII-induced hypertension. In contrast, Nox1 deletion does not improve hypertension in renin transgenics, implying that other mechanisms control chronic hypertension.

In this context, a possible link between Nox1 and vascular inflammation was also investigated. Thrombin increases VSMC Nox1 expression, IL-6 secretion, and NFκB translocation. These latter effects may be mediated by Nox1, as they are inhibited by atorvastatin, which downregulates Nox1. Furthermore, tumor necrosis factor-α or IL-1β induces Nox1-dependent VSMC NFκB activation. Likewise, increased Nox1 expression and activity by hyperhomocysteinemia in rat coronary arteries appear to be mediated by tumor necrosis factor-α. No less important, Nox1 in macrophages is required for foam cell formation, thereby contributing to vascular lesion formation. Therefore, Nox1 appears to mediate vascular inflammation via multiple mechanisms.

Although Nox1 is clearly implicated in vascular pathology, it also has beneficial physiological roles. For example, it is likely responsible for shear-induced outward vessel remodeling, because this response is blocked by deletion of p47phox, but not Nox2.

Figure 1. Spatial and molecular organization of vascular Nox enzymes. Nox1, Nox2, and Nox5 are represented here in different cellular compartments, but can be located either within cells or at the plasma membrane, thus releasing O2− inside vesicles or extracellularly after activation of receptor (R) by ligand (L). O2− may affect cytosolic signaling after crossing membranes via anion channels, reversible protonation, or conversion to H2O2. In contrast, Nox4 is always intracellular and constitutively produces a higher proportion of membrane-permeable H2O2 than other oxidases. All oxidases, except Nox5, form a membrane complex with p22phox. Cytosolic activators vary with oxidase subtype. Rac, p47phox, and Nox4 for Nox2; Poldip2 for Nox4; and Ca2+ for Nox5. Charge compensation mechanisms are unknown, except for VSMC endosomal CIC-3, which supports Nox1 activity. All vascular cells express multiple Nox subtypes simultaneously.
Nox2
Nox2 is found in all vascular wall cells, except VSMC from large arteries, and produces intracellular or extracellular superoxide (Figure 1). Even in large arteries, EC and adventitial fibroblasts are important sources of Nox2-derived superoxide. Nox2 is activated by pathways similar to vascular Nox1 or phagocytic Nox2, such as p47phox phosphorylation by PKC and Src. Similar downstream signals are also affected by Nox1 and Nox2 as described at length in other reviews. For example, Nox2 promotes EC proliferation by p38MAPK and Akt stimulation. On a larger scale, Nox2 also affects vascular function and pathology.

Because O$_2^-$ rapidly neutralizes vasodilator NO, Nox2 is expected to induce contraction. Nox2 expression is inversely correlated with endothelium-dependent relaxation in isolated aorta. Furthermore, Nox2 deletion inhibits PKC-mediated aortic contraction, restores acetylcholine-induced cerebral artery vasodilation, and increases contraction in males. These results suggest that Nox2 may affect blood pressure.

A correlation between Nox2 expression and hypertension has been observed repeatedly. Aortic Nox2 is elevated in stroke-prone spontaneously hypertensive rats, in rats exposed to aldosterone plus salt and in AngII-infused mice. Tempol improves both small intrapulmonary artery Nox2 expression and pulmonary hypertension in renin transgenic rats. As expected, hypertension is worsened by Nox2 overexpression in endothelial-targeted transgens after acute or chronic AngII treatment. In converse experiments, hypertension can be improved by Nox2 deletion, but results depend on the experimental model. Nox2 ablation in mice reduces systemic hypertension in 2-kidney 1-clip or deoxycorticosterone acetate salt animals, and prevents hypoxia-induced pulmonary hypertension. In contrast, Nox2 deletion does not prevent hypertension induced by AngII infusion but decreases medial hypertrophy, affecting blood pressure later. Likewise, hypertension is unaffected in renin transgenic. These results suggest that treatments targeting Nox2 could improve some forms of hypertension.

Accumulating evidence suggests that Nox2 in resident EC and adventitial fibroblasts as well as recruited macrophages promotes inflammation and atherosclerosis. Nox2 mRNA, O$_2^-$ production, and monocyte binding are increased by oscillatory shear stress in EC. Furthermore, in animal models of surgical vascular injury or high-cholesterol diet, Nox2 is significantly upregulated, at least during the chronic phase of the disease, even in the absence of inflammation. Similarly, in human vessels Nox2 expression is correlated

| Table. Examples of Nox1 Signaling in Vascular Smooth Muscle |
|------------------|---------------|-----------------|-----------------|-----------------|--------------------|
| Stimulus         | Upstream Signals | Nox1 Regulation | Downstream Signals | Downstream Effects | References |
| AGE              | Upregulation    | Caveolin phosphorylation, AT1R at plasma membrane |              |                | 23                  |
| Aldosterone      | Upregulation    | P-Akt           | Hypertrophy      | 1, 111           |
| AngII            | Ca$^{2+}$       | PKC Activation | P-p38MAPK        | Hypertrophy      | 111                |
| PKC              | Src             | EGFR            | SHP-2 inactivation |                |                    |
| Rac1             | EGFR            | PI3K            |                 |                |                    |
| bFGF             | PI3K Activation | JNK             | Migration       | 29              |
| PKC              | Rac1            | P-paxillin      | MMP2, MMP9      |                |                    |
| Cyclic stretch   | Upregulation    | NFkB            | Hypertrophy     | 113             |
| IL-1β            | Activation      | MCP-1           | Migration       | 1, 27, 114, 115 |
| LDL oxidized LDL | Upregulation    | SRF-2           |                | 1, 25           |
| PDGF or PGF2α    | PKC5           | EGFR and either | NFkB            | Hypertrophy     | 1, 27, 114, 115 |
| PMA              | Upregulation    |                 | Proliferation   | 1                |
| Serum            | Upregulation    |                 | Proliferation   | 1, 27           |
| Statins          | Downregulation  |                 | Proliferation   | 1                |
| T3               | Upregulation    |                 | Proliferation   | 1, 32           |
| Thrombin         | Upregulation    |                 | Proliferation   | 1                |
| TNF-α            | Activation      | NFkB            |                 | 5                |

EGFR indicates epidermal growth factor receptor; LDL, low-density lipoprotein; TNF, tumor necrosis factor.
with lesion severity, although part of the effect derives from macrophage infiltration in advanced lesions. Moreover, Nox2 deletion greatly reduces descending aortic lesion burden in hyperlipidemic mice. Overall, these results favor a causal role of Nox2 in atherosclerosis.

Nox 4

Nox4 mRNA is present in all vascular wall cells and is significantly more abundant than other Nox enzymes. The protein is located in perinuclear space or endoplasmic reticulum in EC, nucleus in EC and VSMC, and in focal adhesions and stress fibers in VSMC. Because the only regulatory subunit Nox4 shares with Nox1 and Nox2 is p22phox, Nox4 was thought to be constitutively active and responsible for basal ROS production; however, a recent report shows that Poldip2 enhances its activity (Figure 1).

Nox4 is consistently upregulated and acutely activated by transforming growth factor-β in all cell types tested. In fact, transforming growth factor-β is responsible for Nox4 activation in response to other stimuli, such as hypoxia. Investigations of downstream signaling pathways suggest that p38MAPK is a target of Nox4. Nox4 also activates the Ras/ERK pathway, JNK and Akt. In addition to tyrosine kinase signaling pathways, Nox4 has been implicated in the activation of Rho in VSMC, consistent with its effects on the cytoskeleton.

Because there is no report to date of genetic models with altered Nox4 expression, current knowledge derives mostly from interventions in cell culture. Nox4 is linked to decreased vascular cell growth in many studies. For example, whereas serum withdrawal upregulates Nox4 mRNA in VSMC and EC, growth-promoting agents such as AngII, PDGF, IL-1β, thrombin, and phorbol esters downregulate Nox4 mRNA in VSMC and adventitial fibroblasts. However, these results may need to be re-evaluated because of a recent study showing that Nox4 mRNA and protein expressions are not always correlated. Nevertheless, Nox4 is required to maintain the quiescent VSMC phenotype and tumor necrosis factor-α-induced apoptosis in EC. In contrast, Nox4 appears to stimulate growth in other instances. Thus, proliferation induced by urotensin or hypoxia (via transforming growth factor-β) in pulmonary VSMC requires Nox4. Similarly, in EC, Nox4 promotes growth and inhibits apoptosis, and Nox4 knockdown impairs epidermal growth factor-induced and human immunodeficiency virus–tat-induced proliferation. Further studies are required to reconcile these conflicting results.

A similar conundrum holds for the role of Nox4 in migration. Whereas Nox4 siRNA decreases PDGF-induced VSMC migration and wounding-activated EC migration, Nox4 overexpression also decreases migration in PDGF-stimulated VSMC and adventitial fibroblasts exposed to AngII. It is likely that too little Nox4 prevents focal adhesion formation, whereas too much Nox4 prevents focal adhesion dissolution, both of which are required for cell motility.

In some cases Nox4 appears linked to pathways protecting vessels against disease. In EC, Nox4 is upregulated by physiological shear stress and downregulated by pathological stress. Similarly, Nox4 is increased during redifferentiation of smooth muscle after vascular injury. However, Nox4 expression is also correlated with deleterious responses. The proinflammatory mediators tumor necrosis factor-α and oxidized phospholipids increase Nox4 expression or activity, respectively. Basal and urotensin-induced proinflammatory plasminogen activator inhibitor-1 gene expression in pulmonary artery VSMC is dependent on Nox4, and Nox4 down-regulation blocks IL-8, monocyte chemoattractant protein-1, low-density lipoprotein receptor, and ROS production in EC stimulated with oxidized phospholipids. Furthermore, Nox4 is elevated in cerebral aneurysms and in vessels from diabetic mice (except). Of interest, treatment with the peroxisome proliferator-activated receptor-γ agonist rosiglitazone mitigates Nox4 upregulation.

Overall, our knowledge of Nox4 in the vasculature is incomplete. Current evidence suggests that it is involved in numerous essential cellular pathways mediating differentiation, growth, and migration. Its role is still controversial, suggesting that a central aspect of its function remains to be discovered.

Nox5

Nox5 differs from other Nox enzymes by its additional N-terminal regulatory domain. Nox5, which requires no additional subunit, is activated by calcium binding to N-terminal EF-hands (Figure 1). Calcium sensitivity is increased by calmodulin and phosphorylation by PKC. In transfected leukemia cells, Nox5 is activated by H2O2 in a positive feedback loop involving c-Abl. Five splice variants with different tissue distributions are known: Nox5α, Nox5β, Nox5γ, Nox5δ, and Nox5S, a short form without calcium-binding domain. Multiple isoforms are expressed in the vasculature. Within cells, Nox5 can be found in the cytoskeletal fraction, endoplasmic reticulum, or plasma membrane.

Because Nox5 is not present in rodents, experimental models are limited to cultured cells and isolated tissue. In VSMC, Nox5 participates in PDGF-induced proliferation via the JAK/STAT pathway. In EC, Nox5 overexpression slightly increases proliferation and formation of capillary-like tubes, whereas Nox5 depletion reduces thrombin-stimulated growth and tube formation. In intact vessels, adenosine-mediated Nox5 overexpression paradoxically increases endothelial NO synthase activity, but as expected, reduces bioavailable NO via inactivation by O2·−, leading to impaired endothelium-dependent relaxation and increased phenylephrine-induced contraction. One study shows that atherosclerosis increases Nox5 expression and calcium-sensitive oxidase activity in coronary arteries. Nox5 is detected in the endothelium of control arteries, in the neointima of diseased arteries, and in smooth muscle underlying complex lesions in arteries with advanced atherosclerosis.

These studies are provocative, suggesting an important role for Nox5 in vascular function. Clearly, additional work is needed to further explore its physiological and pathological roles.
Interactions Between Oxidases

Because most cells simultaneously express multiple Nox enzymes, some of their functions may be redundant. For example, both Nox2 and Nox4 in EC are required for the angiogenic response and participate in serum-induced proliferation.\textsuperscript{37,41,81} Both enzymes are similarly regulated by shear stress\textsuperscript{56} and colocalize with endoplasmic reticulum markers,\textsuperscript{37} and knockdown of one subtype upregulates the other,\textsuperscript{96} suggesting that their functions are overlapping, although not entirely identical.\textsuperscript{41}

Most often, however, Nox enzymes in the same cell mediate distinct functions. For example, in VSMC from large arteries, Nox1 is required for hypertrophy and proliferation,\textsuperscript{1} whereas Nox4 mediates differentiation.\textsuperscript{60,80} These enzymes

Figure 2. Nox enzymes in multiple tissues contribute to AngII-induced hypertension. AngII infusion increases Nox activity in tissues involved in blood pressure regulation, including brain, kidney, heart, and blood vessels.\textsuperscript{103} Brain Nox controls efferent sympathetic stimulation of lymphoid tissue, leading to activation of T lymphocytes and Nox-dependent upregulation of the CCR5 chemokine receptor and the CD44 hyaluronan receptor. Simultaneously, AngII upregulates the intercellular adhesion molecule-1 (ICAM-1) and the chemokine RANTES in vessels, leading to T-cell migration to vascular periadventitia. AngII and Nox in T cells lead to secretion of tumor necrosis factor-\(\alpha\), which upregulates vascular Nox.\textsuperscript{104,105} In adventitial fibroblasts, VSMC, and EC, Nox generates O\textsubscript{2}\textsuperscript{−}, which depletes NO via conversion to peroxynitrate (ONOO\(^{-}\)), leading to endothelial dysfunction. ROS production is further amplified by ONOO\(^{-}\)-induced NO synthase uncoupling and mitochondrial dysfunction.\textsuperscript{107} Whereas NO induces vasodilation and growth arrest in healthy vessels, O\textsubscript{2}\textsuperscript{−} and H\textsubscript{2}O\textsubscript{2} contribute to vessel contraction and hypertrophy, leading to sustained hypertension. Vascular inflammation produced by mediators such as IL-17 may also induce chronic vascular disease.
respond to different agonists, participate in migration via different mechanisms, and have distinct localizations. Similarly, in transfected HEK293 cells, Nox2 and Nox4 are expressed in different compartments, and each responds to different agonists and activates distinct signaling pathways. Therefore, subcellular localization seems to be an essential factor in determining Nox function.

With regard to vascular disease, multiple NADPH oxidases appear to contribute to the pathophysiological response. Both Nox1 and Nox2 have been implicated in hypertension because they contribute directly to blood pressure elevation and to end-organ damage or remodeling. The expression of Nox1, Nox2, and Nox4 is altered after vascular injury, with the former two mediating the proliferative response of VSMC and EC, and the latter most likely contributing to redifferentiation of VSMC. In diabetic vascular disease, all 3 are upregulated, however, their specific contributions to diabetes-related endothelial dysfunction and inflammation remain to be elucidated.

It is also important to consider that Nox enzymes in multiple tissues contribute to the pathological response in a coordinated fashion. In hypertension, evidence exists for a role of brain, vascular, cardiac, and kidney Nox enzymes. Furthermore, a large part of the hypertensive response to AngII infusion or deoxycorticosterone acetate salt administration is dependent on T lymphocytes. Harrison and Gongora have proposed that stimulation of Nox enzymes in circumentricular organs by AngII increases efferent sympathetic activity, leading to activation of T cells in lymph nodes, which home to vessels and kidneys. Importantly, T cells release cytokines that stimulate NADPH oxidases in these tissues, causing vasoconstriction and sodium retention (Figure 2). A similar coordinated response is likely to occur in the vessel wall in atherosclerosis, where infiltrated macrophages release cytokines in a Nox2-dependent manner, which then activate Nox1 and Nox2 in VSMC, EC, and adventitial fibroblasts.

Once ROS production is initiated by Nox activation, other oxidase systems often amplify this initial response. For example, EC exposed to pathological shear stress exhibit a Nox-dependent activation of xanthine oxidase. Similarly, AngII stimulation of these cells enhances mitochondrial ROS production in a Nox-dependent manner. In hypertension, Nox activation leads to the formation of peroxynitrite, which uncouples endothelial NO synthase, leading to a dramatic enhancement of \( \text{O}_2^- \) production. Moreover, ROS can also activate Nox enzymes and increase their own production in a positive feedback loop (Figure 2). Such self-reinforcing loops may set new homeostatic points of ROS production and lock the organism in a sustained pathological state.

Conclusion

The evidence summarized in this review clearly shows that Nox enzymes are essential to normal vascular function and participate in the development of vascular disease. They modulate intracellular signaling pathways to regulate cell survival, vessel contraction, and relaxation. In pathological conditions, they contribute to VSMC proliferation and migration, angiogenesis, inflammation, hypertension, and medial hypertrophy. Because of their varied and essential functions, it is not surprising that antioxidants designed to scavenge ROS produced from these enzymes provide ineffective protection against vascular disease. The work described here strongly argues for a change in therapeutic strategies to target only deleterious sources of ROS while leaving intact those that are required for normal vascular function.

That said, more research is necessary to fully understand the roles of different Nox enzymes in the vasculature. Additional studies should focus on identifying the specific molecular pathways that are targets of each Nox. The creation of tissue-specific, genetically modified animals will permit a better analysis of their roles in physiology and disease. Moreover, subtype-specific inhibitors are needed to allow the field to advance therapeutically once pathological targets have been identified. Studies such as these will allow us to truly test the oxidative hypothesis of vascular disease.

Sources of Funding

This work was supported by National Institutes of Health grants HL38206, HL058863, and HL095070.

Disclosures

Emory University is the owner of a patent on Nox1 with both authors cited in the list of inventors.

References


NADPH Oxidases: Functions and Pathologies in the Vasculature
Bernard Lassègue and Kathy K. Griendling

Arterioscler Thromb Vasc Biol. 2010;30:653-661; originally published online November 12, 2009;

doi: 10.1161/ATVBAHA.108.181610

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/30/4/653

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/