Editorial

Inflammation and Vascular Hypertrophy Induced by Angiotensin II
Role of NADPH Oxidase-Derived Reactive Oxygen Species Independently of Blood Pressure Elevation?

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Evidence from the last few years has suggested that increased oxidative stress plays a pathophysiological role in cardiovascular disease, including atherosclerosis, hypertension, and heart failure. At the same time, emerging data have implicated inflammation as a process involved in the initiation and progression of atherosclerosis, but it is also present in hypertension, diabetes mellitus, and other conditions associated with vascular damage. A large body of data has suggested that the renin-angiotensin system mediates part of its physiological and pathophysiological actions via generation of reactive oxygen species (ROS) and stimulation of inflammation.

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Liu et al. show that angiotensin II (Ang II)–enhanced NADPH oxidase activity contributes to adhesion molecule (ICAM) expression, leukocyte infiltration, and vascular growth in rats, independently of its effects on blood pressure. These important findings expand our knowledge on some of the pleiotropic actions of Ang II, and the mechanisms whereby Ang II–induced inflammation and growth may be mediated by ROS.

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ROS, which include superoxide anion and hydrogen peroxide among others, are signaling molecules that participate in the regulation of the tone and structure of blood vessels. Superoxide anion in the vascular wall may be produced primarily in the endothelium and the adventitia as well as in vascular smooth muscle cells and fibroblasts. The sources of ROS, and specifically of superoxide anion, have been the subject of debate and numerous studies in the past few years, and major strides have been made in identifying the mechanisms of their generation. Several enzymes such as nitric oxide synthase, xanthine oxidase, and myeloperoxidase may generate free radicals, but in the vasculature it appears that the most important enzyme responsible for superoxide anion generation is NAD(P)H oxidase. The production of superoxide anion occurs when oxygen is reduced by an electron with NAD(P)H functioning as the electron donor. NAD(P)H oxidase was originally found in neutrophils. The neutrophil/macrophage NAD(P)H oxidase is composed of five subunits: p40phox (phox for PHagocyte OXidase), p47phox, p67phox, p22phox, and gp91phox (Figure).

Activation requires the participation of two low–molecular weight guanine nucleotide-binding proteins, Rac2 (or Rac1) and Rap1A.

It has become increasingly evident that subunits of the leukocyte NAD(P)H oxidase system are also present in nonphagocytic cells, including cells of the vasculature. All subunits of the neutrophil NAD(P)H oxidase are found in endothelial cells. Of critical importance in the generation of endothelial-derived superoxide are gp91phox and p47phox, as well as p22phox, p22phox, p47phox, and p67phox have also been detected in adventitial fibroblasts. The situation in vascular smooth muscle cells appears to be more complicated, and it is still unclear which of the neutrophil NAD(P)H oxidase subunits are present and functionally active in these cells. In rat conduit arteries, mRNA for gp91phox is barely detectable in smooth muscle cells. However, homologues of gp91phox, nox1, and nox4 (for Nonphagocytic NADPH Oxidase) as well as p22phox, p47phox, and rac1, are expressed in rat aortic smooth muscle cells (Figure). Although initial studies suggested that nox1 is a subunit-independent low–capacity superoxide-generating enzyme involved in the regulation of mitogenesis, recent data indicate that nox1 requires p47phox and p67phox and that it is regulated by NoxO1 (Nox organizer 1) and NoxA1 (Nox activator 1). In vascular smooth muscle cells derived from human resistance arteries, we recently demonstrated that gp91phox and nox4 are present, whereas nox1 is undetectable. Furthermore gp91phox plays a major role in superoxide production in these cells.

Mechanisms of vascular NAD(P)H oxidase stimulation by Ang II and the potential implication of NAD(P)H oxidase-derived ROS in signaling in vascular cells have received considerable attention. Ang II via AT1 receptors may stimu-
However, use of the peptide inhibitor may be superior to by gp91ds, which unfortunately was not done in this study. (scrambled gp91ds) to ensure the specificity of the blockade to use a control that includes a scrambled docking sequence logues are targeted by the inhibitor. It would have been useful which is internalized by all late PKC, PLD, or Src\textsuperscript{21,22} to activate NAD(P)H oxidase. In turn, superoxide anion generated by NAD(P)H oxidase will stimulate JNK and p38 MAP kinase, which will mediate the effects of increased oxygen free radicals.\textsuperscript{23,24} In the study of Liu et al\textsuperscript{5} in this issue, the involvement of activation of NAD(P)H oxidase is shown to lead to growth and inflammation. In brief, an inhibitor of the nox subunit of NADPH oxidase (gp91ds-tat peptide) co-infused with Ang II into rats resulted in reduction in the increased production of superoxide (as measured by formation of nitrotyrosine), inflammation (infiltration of macrophages), upregulation of inflammatory mediators (ICAM-1), and growth of the wall of the aorta, in absence of blood pressure lowering. The authors conclude that stimulation by Ang II of endothelial and adventitial NADPH-derived ROS results in an inflammatory and remodeling vascular response in rats that is independent of blood pressure. The importance of this article lies in the clear-cut demonstration of a NAD(P)H oxidase-mediated effect on growth and inflammation in vivo by use of a specific selective inhibitor of the enzyme. In fact, the inhibitor used is quite ingenious. It is based in the use of short amino acid sequences involved in the docking of phosphorylated p47phox to gp91phox (hence the name gp91docking sequence or gp91ds). These are then coupled to a 9-amino acid peptide from the HIV viral coat “tat” which is internalized by all cells, allowing the gp91ds-tat to reach and inhibit gp91phox with reasonable chance of being highly specific. A limitation of the study is that the selectivity of this inhibitor has not been definitively demonstrated to be absolute, and studies using inhibitors almost always leave such questions unanswered. Furthermore, it is unclear which of the specific nox homologues are targeted by the inhibitor. It would have been useful to use a control that includes a scrambled docking sequence (scrambled gp91ds) to ensure the specificity of the blockade by gp91ds, which unfortunately was not done in this study. However, use of the peptide inhibitor may be superior to others, such as apocynin, and certainly provides greater understanding of mechanism than the use of an antioxidant.

Liu et al\textsuperscript{5} attribute the ROS formation in response to Ang II to endothelium and adventitial production. However, the media smooth muscle cells which are known to possess functionally active nox-containing NAD(P)H oxidase and where significant staining for nitrotyrosine could be observed may also have participated in the response described. Furthermore, neutrophils and macrophages that infiltrated the adventitia may have also functioned as an important source of NAD(P)H-derived ROS that would be inhibited by gp91ds-tat. Thus, the source of ROS is not unambiguously demonstrated by the study, and the differences in degree of upregulation of ICAM cited by the authors are not definitive proof of the site of generation of ROS.

Together with previous data showing the proinflammatory effect of Ang II-induced ROS leading to NF-κB and AP-1 upregulation,\textsuperscript{3,25,26} and recent publications, which, by using DNA microarray technology, identified the genes stimulated in response to Ang II,\textsuperscript{27} demonstrating upregulation of inflammatory mediators such as osteopontin, PAI-1, MCP-1, and tissue factor, the present study significantly contributes to our knowledge of the pleiotropic actions of Ang II that participate in the pathophysiology of cardiovascular disease.

References

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