Angiotensin II–Stimulated Vascular Remodeling

The Search for the Culprit Oxidase

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Hypertrophy and hyperplasia of vascular smooth muscle cells are hallmarks of the common vascular disorders of atherosclerosis, restenosis, and hypertension and contribute to their long-term sequelae. Angiotensin II (Ang II) is a potent smooth muscle mitogen and hypertrophic agent. The importance of Ang II in the pathogenesis of vascular disease is reflected in the efficacy of angiotensin-converting enzyme inhibitors and Ang II receptor blockers in the treatment of atherosclerosis and hypertension. Despite the widespread use of these agents in clinical practice, our understanding of the mechanisms through which Ang II exerts its effects on the vasculature is not complete.

The studies by Lassègue et al\(^1\) and Wang et al\(^2\) in this issue of Circulation Research go a long way in elucidating the molecular basis for the effects of Ang II on vascular smooth muscle cell growth.

To fully appreciate the significance of the reported findings, one has to place them into historical perspective. The role of oxidative stress in the pathogenesis of the above-mentioned vascular disorders has been well recognized for some time.\(^3\) It has come to light that humoral factors, such as Ang II, platelet-derived growth factor, and thrombin, directly lead to oxidative stress in smooth muscle cells via the generation of reactive oxygen species (ROS), which are essential for their mitogenic and hypertrophic properties.\(^4\)\(^\text{-}\)\(^7\) With these findings in hand, investigators directed their efforts toward identifying the enzymatic source of growth factor–stimulated ROS in vascular smooth muscle cells.

Attention was mainly focused on identifying an oxidase functionally analogous to the phagocyte NADPH oxidoreductase, because many but not all components of its multimolecular complex are expressed in vascular smooth muscle cells.\(^7\)\(^8\) However, attempts to show significant expression of the enzymatically active flavoprotein subunit gp91\(^\text{phox}\) of this oxidase in smooth muscle cells were unsuccessful. Therefore, the cloning of a homologous protein nox1 (NAD(P)H oxidase 1), originally termed mox-1 (mitogenic oxidase-1), from a colon cancer cell line, and also expressed to a lesser extent in rat vascular smooth muscle cells, was hailed as a significant breakthrough.\(^9\) Other gp91\(^\text{phox}\) homologues, expressed primarily in the kidney\(^10\) and thyroid,\(^11\) have also been identified recently.

This brings us to the present study by Lassègue et al,\(^1\) demonstrating for the first time the importance of the newly discovered nox1 in Ang II–stimulated and platelet-derived growth factor–stimulated short-term ROS generation and activation of the growth-promoting signaling proteins Akt and p38 mitogen-activated protein kinase in cultured rat smooth muscle cells. In the absence of specific means to inhibit nox1 activity, Lassègue et al use an adenovirus encoding antisense nox1 to suppress its expression. The study also suggests that transcriptional upregulation of nox1 by Ang II may be responsible for longer-term growth of smooth muscle cells induced by this mitogen. Standing alone, the study provides convincing evidence for the role of nox1 in mitogen-stimulated smooth muscle cell growth in vitro. However, on the basis of the methodology used, it would be fair to say that the data are not conclusive in this regard. Antisense, though effective at suppressing nox1 expression, may not be entirely specific. This leaves open the possibility, which is acknowledged by the authors, that another homologous oxidase, known or unknown, may also participate in mitogen-stimulated ROS generation and growth.

The study by Wang et al,\(^2\) not coincidentally published in this same issue, seems to contradict the in vitro findings of Lassègue et al\(^1\) or at least question their physiological relevance. Using knockout mice, Wang et al\(^2\) prove that it is gp91\(^\text{phox}\), present primarily in endothelial cells and adventitial fibroblasts but also expressed to a much lesser degree in smooth muscle cells, that is chiefly responsible for Ang II–stimulated vascular oxidative stress and smooth muscle growth in vivo. This supports a previous report that Ang II stimulates ROS production in adventitial fibroblasts by inducing other components of the NADPH oxidase.\(^12\) Assuming for the moment that there is a mouse homologue of nox1 (one has yet to be cloned) and ignoring possible species-specific differences in the response to Ang II, there are at least two explanations for the seemingly contradictory findings embodied in these studies. The most straightforward one is that nox1 is not expressed in smooth muscle cells in vivo or that expressed nox1 plays a small role, if
any, in smooth muscle hypertrophy and hyperplasia in response to Ang II in vivo. On the contrary, it is gp91phox, albeit expressed at very low levels in smooth muscle cells, that mediates the effects of Ang II.

Another scenario, and one that is much more likely, is that Ang II–stimulated, ROS-regulated smooth muscle growth in vivo is not simply a function of ROS generated by Ang II in smooth muscle cells, whether that be through gp91phox, nox1, or other oxidases. Rather, in the complex milieu of the vascular wall, smooth muscle growth is dependent on the net balance between intracellularly generated ROS (in smooth muscle cells), diffusible ROS produced in adjacent cells in the endothelium and adventitia, and, perhaps most importantly, diffusible, endotheli-um-derived nitric oxide (NO), a potent inhibitor of smooth muscle hypertrophy and growth.13 In such an environment, an increase in ROS (particularly diffusible and membrane-permeable superoxide-derived H₂O₂) through gp91phox in the endothelium and adventitia would, through direct or indirect actions, lead to smooth muscle cell growth (Figure). These actions could include, but are not limited to, counteracting the tonic inhibition on smooth muscle cells by decreasing bioavailable endothelium-derived NO,14 direct stimulation of smooth muscle mitogenesis and hypertrophy,15,16 elaboration of smooth muscle mitogens,17 or changing mitogen-receptor affinity.18

It is important to note that the regulation of Ang II–induced smooth muscle proliferation or hypertrophy in vivo by endothelial or adventitial gp91phox does not exclude the expression of nox1 in medial smooth muscle cells nor does it imply that Ang II does not lead to activation or upregulation of nox1 in vivo. It does suggest, however, that in a whole vessel, more powerful forces overshadow the likely effect of nox1-derived ROS on medial hypertrophy: ROS and NO derived from the endothelium and adventitia. To definitively demonstrate a role for nox1 in medial growth in a physiological setting will require the generation of nox1-null animals.

In summary, both the above-mentioned studies are significant advances toward understanding the molecular pathogenesis of Ang II–stimulated arterial remodeling. However, our enthusiasm to embrace these findings should be tempered by the reminder that our present understanding of and ability to replicate the complex interactions between cells comprising the vascular wall is still quite rudimentary. Thus, cellular and even animal models of this disease may not be accurate reflections of the human condition.

References


**Key Words:** NAD(P)H oxidase ■ angiotensin II ■ smooth muscle ■ medial hypertrophy ■ reactive oxygen species
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