No Superoxide—No Stress?
Nox4, the Good NADPH Oxidase!

Ralf P. Brandes, Ina Takac, Katrin Schröder

ADPH oxidases of the Nox family are enzymes whose only known function is the production of reactive oxygen species (ROS). Since the first report on a vascular NADPH oxidase by Griendling et al in 1994, several thousand publications have been devoted to this topic. The general impression is that under physiological conditions, Nox proteins contribute to vascular signaling, whereas the overactivation and induction of NADPH oxidases promote vascular disease.

In the vascular system, the NADPH oxidases Nox1, Nox2, Nox4, and Nox5 are expressed. Unlike the case with the other vascular Nox homologues, the activity of Nox4 appears to be predominantly controlled by its expression level, and proinflammatory mediators that induce Nox1 or Nox2 instead appear to suppress Nox4 expression. Nox4 is also the only vascular homolog that directly produces hydrogen peroxide (H₂O₂) and thus is incapable of scavenging nitric oxide (NO) or producing peroxynitrite (ONOO⁻). As a consequence of these unique properties, so far little consensus regarding the physiological function of Nox4 has been reached.

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Ray et al report the generation and characterization of a transgenic mouse with endothelial-specific Nox4 overexpression. Vascular segments and endothelial cells of these animals had a significant increase in H₂O₂ generation that was sufficient to result in a substantial increase in the oxidation of peroxiredoxin 1. Despite these signs of increased protein oxidation, the blood pressure of the animals was lower under basal conditions and after angiotensin II treatment. Furthermore, endothelium-dependent relaxation was enhanced compared with wild-type animals. The latter effects were sensitive to catalase ex vivo and demonstrated that Nox4 increases the fluorescent signal in the dihydroethidium assay but that this is not a consequence of an H₂O₂-mediated direct oxidation of dihydroethidium to oxyethidium and therefore O₂⁻ generation on Nox4 overexpression. In contrast to this, several studies reported O₂⁻ formation, did not result in smooth muscle cell hypertrophy. This observation is remarkable given that hypertrophy is readily achieved by smooth muscle–targeted NADPH oxidase overexpression or direct application of H₂O₂. It is possible that the hyperpolarization induced by endothelium-derived H₂O₂ counteracted hypertrophic signaling in the study by Ray et al. Alternatively, it may illustrate that H₂O₂ signaling is much more compartmentalized than had long been assumed. Indeed, evidence is accumulating that locally produced H₂O₂ rapidly reacts with peroxiredoxins, which subsequently mediate signaling by thiol-based redox reactions, although a lot of work still has to be done to establish this pathway as a central intermediate in Nox-derived redox signaling.

Interpretation of the physiological function of Nox4 critically depends on the inability of the enzyme to produce O₂⁻, and Ray et al support this view, as they found no evidence of increased tyrosine nitration, a footprint marker of ONOO⁻ formation and therefore O₂⁻ generation on Nox4 overexpression. In contrast to this, several studies reported O₂⁻ formation by Nox4, but these data were mainly based on microscopy fluorescence measurements with the O₂⁻-sensitive probe dihydroethidium. In the present work, Ray et al impressively demonstrate that Nox4 increases the fluorescent signal in the dihydroethidium assay but that this is not a consequence of an O₂⁻-dependent formation of oxyethidium but rather of a H₂O₂-mediated direct oxidation of dihydroethidium to ethidium, a reaction that is in fact completely sensitive to catalase. Such caveats for the microscopy evaluation of the
The dihydroethidium assay have been previously presented by others, too.11 The conclusion of these observations can only be that fluorescence microscopy should no longer be used to evaluate the dihydroethidium assay and that it should be accepted that Nox4 indeed primarily produces H2O2.

An important conclusion of the present work by Ray et al5 could be that Nox4 serves protective functions (Figure), which is in line with the ability of Nox4 to prevent the transition of cardiac hypertrophy to heart failure by maintaining vascular endothelial growth factor–induced angiogenesis.12 These findings are contrasted by observations identifying Nox4 as mediator of heart failure after transaortic constriction13 and of epithelial cell death in the lung.14 How can these findings be reconciled? Despite the beneficial signaling role of H2O2 in the endothelium, it is still true that excessive concentrations of this oxidant induce inflammation, fibrosis, apoptosis, and even necrosis. Given the rather constitutive activity of Nox4, the regulation of H2O2 formation by the enzyme occurs predominantly on the expression level. Thus, whether Nox4 is harmful or beneficial is primarily a question of dosage. Under certain conditions, such as the release of transforming growth factor-β1, diabetes, and heart failure, Nox4-dependent H2O2 formation appears to exceed protective levels and even becomes harmful. This is reminiscent of other enzymes involved in redox signaling. Low NO, for example, is beneficial, whereas excessive formation by inducible NO synthase can have harmful effects as well.

One unexpected finding in the present study of Ray et al5 is that endothelial overexpression of Nox4 improved the stimulated endothelium-dependent relaxation in response to acetylcholine and histamine. This implies that these 2 agonists may enhance Nox4 activity, which is unexpected given the high basal, so-called constitutive activity of the enzyme, which is consistently observed in overexpression systems. In line with the present observations, several reports suggested that despite the high constitutive activity of the enzyme, transforming growth factor-β1 in particular may acutely further increase Nox4 activity. It is unclear whether this effect involves translocation or phosphorylation of the enzyme, changes in unknown activating factors such as interacting proteins or lipids, or even the availability of the substrate NADPH.2

In conclusion, with the present work, Ray et al5 indicate that Nox4 in the endothelium can have beneficial effects by mediating an EDHF-type endothelium-dependent vasodilatation, which is sufficient to systemically lower the blood pressure. This work impressively illustrates that the site and type of ROS generated by Nox proteins are decisive for their function. Future work in Nox4 knockout mice, however, will be required to uncover whether the positive effects elicited by overexpression are also exerted by endogenous Nox4.

**Sources of Funding**
This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB815/TP1 and SFB834/TPA2) and the Excellence Cluster Cardiopulmonary System.
References


**Key Words:** endothelial function ■ endothelium ■ hypertension ■ nitric oxide ■ reactive oxygen species
No Superoxide—No Stress?: Nox4, the Good NADPH Oxidase!
Ralf P. Brandes, Ina Takac and Katrin Schröder

Arterioscler Thromb Vasc Biol. 2011;31:1255-1257
doi: 10.1161/ATVBAHA.111.226894
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 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/31/6/1255

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/