NO or H₂O₂ for Endothelium-Dependent Vasorelaxation
Tetrahydrobiopterin Makes the Difference

Victor W.M. van Hinsbergh

Rapid adaptation of the diameter of blood vessels is an important regulatory mechanism for regulating blood pressure and for limiting tissue damage resulting from wounding or ischemia. Endothelial cells contribute to vasoregulation by the production of potent vasodilators. Loss or reduction of endothelium-dependent vasorelaxation is one of the reflections of endothelial dysfunction, an early hallmark in the development of atherosclerosis. Studies in patients have pointed to an impairment of endothelium-dependent vasodilation in a number of conditions that also increase the risk of developing cardiovascular complications, including hypercholesterolemia, hypertension, and diabetes.1-4 This impairment is due to a reduced availability of NO, a major endothelium-dependent vasorelaxing factor.5,6 Several studies have indicated that supplementation of tetrahydrobiopterin (H₄B), a cofactor of the endothelial NO synthase, can improve endothelium-dependent arterial vasoregulation in atherosclerotic and diabetic patients.7-10 In the April issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Cosentino et al11 use transgenic mice to achieve a 60% reduction of arterial H₄B, and they demonstrate that this reduction is accompanied by a marked decrease of NO production while H₂O₂ production is increased.

See page 496

Endothelial cells produce NO from L-arginine by NO synthase-3 (NOS-3), also called constitutive or endothelial NOS. The enzyme is constitutively present in endothelial cells and becomes active by interaction with the Ca²⁺/calmodulin complex, which is transiently generated after exposure of the endothelial cell to vasoactive agents, or by phosphorylation at serine 1117 by the serine/threonine protein kinase Akt.12,13 Arterial shear forces and estrogen are among the factors that can activate NOS-3 via Akt. NOS-3 consists of 2 enzymatic domains, a flavin-containing reductase domain and a heme-containing oxygenase domain. These domains are connected by a regulatory calmodulin-binding domain. Binding of the Ca²⁺/calmodulin complex orients the other domains in such a position that NADPH-derived electrons generated on the reductase domain flow to the oxygenase domain.12 Phosphorylation at position Ser1117 probably causes a comparable effect.13 The oxygenase domain of NOS-3 contains an iron ion (Fe³⁺) that binds oxygen on reduction (Fe²⁺), and this complex finally causes the conversion of L-arginine to NO and L-citrulline. This sequence of events occurs adequately only if the cofactor H₄B is bound to the NOS-3 protein.14,15 On the basis of structural analysis, it has been concluded that H₄B does not directly participate in the oxygen activation reactions that are critical for NO formation but that it probably has a function in the stabilization of the enzyme.16 In the absence of H₄B, the NOS-3 enzyme is “uncoupled” and produces mainly superoxide instead of NO, as became clear from electron spin resonance studies.17,18 In this case, the intermediate Fe²⁺-O₂ complex dissociates and forms superoxide (O₂⁻) and the original Fe³⁺ group of the NOS-3 protein.

Endothelium-dependent vasodilation can also be induced by other mediators. Prostacyclin and prostaglandin E₂ induce vasodilation in specific regions of the circulatory system. Furthermore, endothelium-derived hyperpolarizing factors (EDHF) play a major role in the vasoregulation of intramyocardial arteries/arterioles. In endothelial NO synthase-deficient mice, endothelium-dependent vasoregulation is largely taken over by EDHFs.19 Products generated by cytochrome P-450 monoxygenase activity, in particular, epoxyeicosatrienoic acids, have been identified as EDHFs.20 The cytochrome P-450 subtype CYP9 was identified as a major contributor to EDHF generation.21 Two recent studies have indicated that hydrogen peroxide (H₂O₂) is also an EDHF.11,22 Matoba et al22 showed that H₂O₂ elicited hyperpolarization of smooth muscle cells and vasorelaxation of small mesenteric arteries in the mouse. Cosentino et al11 now provide evidence that under conditions of H₂B deficiency, H₂O₂ instead of NO acts as an endothelium-derived relaxing factor in the mouse aorta and that NOS-3 is an important source of this EDHF. Although previous studies have shown that H₂O₂ is able to induce vasorelaxation by activating soluble guanylate cyclase in smooth muscle cells,23,24 the present studies provide first evidence that it indeed acts as such in a physiological setting.

The discovery that H₂O₂ generated by uncoupled NOS-3 is active in endothelium-dependent vasorelaxation comes from an elegant series of experiments in which Cosentino et al11 used H₂B-deficient mice to alter the activity of NOS-3. Given the importance of the H₂B for proper functioning of NOS-3, these authors considered using a mouse model of hyperphenylalaninemia, the hph-1 mouse. This mouse displays a 90% deficiency in the activity of GTP cyclohydrolase I, a key enzyme in the de novo H₂B synthesis. As a consequence, these animals have a 60% reduction of H₂B in their aortas. The residual H₂B is probably generated by the so-called salvage pathway, in which sepiapterin is the substrate for H₂B

From the Gaußius Laboratory, Leiden, and the Department of Physiology, Institute for Cardiovascular Research, Vrije Universiteit Medical Center, Amsterdam, the Netherlands.

Correspondence to Dr Victor W.M. van Hinsbergh, Gaußius Laboratory TNO-PG, Zernikedreef 9, 2301 CE Leiden, Netherlands. E-mail vwm.vanhinsbergh@pg.tno.nl


© 2001 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org
synthesis. The hph-1 mice exhibited a normal blood pressure and a normal vasoconstrictive response to norepinephrine. Unexpectedly, the endothelium-dependent vasorelaxation on acetylcholine stimulation of preconstricted aortas was normal. This response was not due to NO production, which was markedly impaired by the H4B deficiency, but to H2O2. The endothelium-derived vasorelaxation induced by acetylcholine in the aortas of hph-1 mice was reduced by catalase, the enzyme that scavenges H2O2. This is in contrast to the situation in wild-type animals, which mainly produced NO as an endothelium-derived vasorelaxant. The vasodilation in hph-1 mice was completely inhibited by NADPH oxidase. The increase, but the data show the reverse. The authors suggest because of the uncoupling of NOS-3, superoxide would oxidize anions can be detected in the hph-1 mouse aorta than in the endothelial H4B status and NO production. 33 Because H4B deficiency provides an interesting clinical perspective. 33 However, whether this is directly related to the altered mode of action of NO production or to a complex formation between NOS-3 and superoxide dismutase or whether enzymatic activities are induced in the endothelium of hph-1 mice may cause vascular damage at advanced age. If this were the case, partial H4B deficiency, such as is seen in various groups of patients prone to cardiovascular disease, would not only cause reduced efficacy of endothelium-dependent vasoregulation but would also increase endothelial damage in due course. Even if defense mechanisms are appropriate in the healthy vessel, an increased H2O2 production in dysfunctioning endothelial cells may accelerate the progression of vascular disease. If such a mechanism plays a role, improvement of the H4B status of the endothelium would give long-term benefits for the vessel wall by improving NO generation and by reducing H2O2-mediated damage in the endothelium. A systematic long-term evaluation of dietary suplementations that improve the vascular H4B status of humans and the presence of multiple cell layers in the thickened intima, which are not involved in vasodilatation but certainly are able to interact with and modify reactive oxygen species, make it difficult to predict its efficacy. Another issue to be resolved is the effect of enhanced endothelial H2O2 generation on the long-term function of the vascular endothelium. The investigators used young animals (aged 8 weeks) for their studies. Because H2O2 generates the highly reactive hydroxyl radical in the Fenton reaction, which is generally considered as very damaging, one wonders whether the increased production of H2O2 by NOS-3 in H4B-deficient mice may cause vascular damage at advanced age. If this were the case, partial H4B deficiency, such as is seen in various groups of patients prone to cardiovascular disease, would not only cause reduced efficacy of endothelium-dependent vasoregulation but would also increase endothelial damage in due course. Even if defense mechanisms are appropriate in the healthy vessel, an increased H2O2 production in dysfunctioning endothelial cells may accelerate the progression of vascular disease. If such a mechanism plays a role, improvement of the H4B status of the endothelium would give long-term benefits for the vessel wall by improving NO generation and by reducing H2O2-mediated damage in the endothelium. A systematic long-term evaluation of dietary suplementations that improve the vascular H4B status in various types of patients at risk for developing vascular complications is urgently required.

References


**KEY WORDS:** nitric oxide synthase □ hydrogen peroxide □ superoxide anion □ catalase GTP cyclohydrolase I
NO or $\text{H}_2\text{O}_2$ for Endothelium-Dependent Vasorelaxation: Tetrahydrobiopterin Makes the Difference

Victor W. M. van Hinsbergh

*Arterioscler Thromb Vasc Biol.* 2001;21:719-721
doi: 10.1161/01.ATV.21.5.719

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 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/21/5/719

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/