Editorial

NO or H$_2$O$_2$ 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. This impairment is due to a reduced availability of NO, a major endothelium-dependent vasorelaxing factor. Several studies have indicated that supplementation of tetrahydrobiopterin (H$_4$B), a cofactor of the endothelial NO synthase, can improve endothelium-dependent arterial vasoregulation in atherosclerotic and diabetic patients.

In the April issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Cosentino et al now use transgenic mice to achieve a 60% reduction of arterial H$_4$B, and they demonstrate that this reduction is accompanied by a marked decrease of NO production while H$_2$O$_2$ production is increased.

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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 Ca$^{2+}$/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. 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 Ca$^{2+}$/calmodulin complex orients the 1$^\text{st}$-O$_2$ complex that binds oxygen on reduction (Fe$^{3+}$), 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 H$_4$B is bound to the NOS-3 protein.

On the basis of structural analysis, it has been concluded that H$_4$B 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. In the absence of H$_4$B, the NOS-3 enzyme is “uncoupled” and produces mainly superoxide instead of NO, as became clear from electron spin resonance studies. In this case, the intermediate Fe$^{2+}$-O$_2$ complex dissociates and forms superoxide (O$_2^-$) and the original Fe$^{3+}$ group of the NOS-3 protein.

Endothelium-dependent vasodilation can also be induced by other mediators. Prostacyclin and prostaglandin E$_2$ induce vasodilation in specific regions of the circulatory system. Furthermore, endothelium-derived hyperpolarizing factors (EDHFs) 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. Products generated by cytochrome P-450 monooxygenase activity, in particular, epoxidecysatrienoic acids, have been identified as EDHFs.

The cytochrome P-450 subtype CYP9 was identified as a major contributor to EDHF generation. Two recent studies have indicated that hydrogen peroxide (H$_2$O$_2$) is also an EDHF. Matoba et al showed that H$_2$O$_2$ elicited hyperpolarization of smooth muscle cells and vasorelaxation of small mesenteric arteries in the mouse. Cosentino et al now provide evidence that under conditions of H$_2$B deficiency, H$_2$O$_2$ 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 H$_2$O$_2$ is able to induce vasorelaxation by activating soluble guanylate cyclase in smooth muscle cells, the present studies provide first evidence that it indeed acts as such in a physiological setting.

The discovery that H$_2$O$_2$ generated by uncoupled NOS-3 is active in endothelium-dependent vasorelaxation comes from an elegant series of experiments in which Cosentino et al used H$_2$B-deficient mice to alter the activity of NOS-3. Given the importance of the H$_2$B 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 H$_2$B synthesis. As a consequence, these animals have a 60% reduction of H$_2$B in their aortas. The residual H$_2$B is probably generated by the so-called salvage pathway, in which sepiapterin is the substrate for H$_2$B
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 N^2-monomethyl-L-arginine, which directly couples the generation of H2O2 to the production of reactive oxygen species, in particular, superoxide anion by NOS-3. Conversion of superoxide anions in H2O2 by an exogenous supply of superoxide dismutase increased vasodilation in the hph-1 mice. The study of Cosentino et al leads to 2 important conclusions. First, it adds firm experimental evidence to the suggestion that H4B deficiency impairs NO production by NOS-3. An adequate NO production is not only important for vasorelaxation; NO is also considered to be a protective factor that counteracts atherosclerosis. Furthermore, NO is involved in the prevention of platelet activation, counteracting inflammatory functions of the endothelium, including the expression of leukocyte adhesion molecules and chemokines, the regulation of the endothelial barrier function, and angiogenesis.25–28 Second, the study shows that in healthy aortas of young hph-1 mice, acetylcholine-dependent vasorelaxation can be caused by H2O2; thus, it recognizes H2O2 as a potential and pathophysiologically relevant endothelium-derived mediator of vasorelaxation.

The study of Cosentino et al11 indicates that fewer superoxide anions can be detected in the hph-1 mouse aorta than in the wild-type mouse aorta. One might have anticipated that because of the uncoupling of NOS-3, superoxide would increase, but the data show the reverse. The authors suggest that superoxide is converted to hydrogen peroxide. The mechanism by which this conversion occurs is not yet clear. Apparently, H4B deficiency induces the increased conversion. However, whether this is directly related to the altered mode of action of NOS-3 or to a complex formation between NOS-3 and superoxide dismutase or whether enzymatic activities are induced in the endothelium of hph-1 mice because of the increased demand of H2O2 generation remains to be clarified. Heme oxygenase (HO)-2 may be a candidate to play a role in such a process, because both HO-2 and NOS-3 are constitutively present in endothelial cells, and HO-2 activity is inhibited by NO.29,30 HO activity induces manganese superoxide dismutase in astrocytes.31 Therefore, it would be interesting to know whether HO activity could also induce superoxide dismutase activity in endothelial cells.

The recognition of H4B as a crucial cofactor for proper NOS-3 activity provides an interesting clinical perspective. Several studies have shown that supplementation of H4B or 5-methyltetrahydrofolate, an active folate form that is thought to be involved in H4B regeneration, can indeed improve endothelium-dependent vasodilation in patients with atherosclerosis, coronary artery disease, and type II diabetes.7–10,32 Furthermore, vitamin C stabilizes H4B and thus may improve the endothelial H4B status and NO production.33 Because H4B and folate can be given orally as a nutrient, these data reveal an important new potential target for preventive strategies for countering cardiovascular complications.

Several additional questions come from the study of Cosentino et al.11 In the mouse study, supplementation of H4B improved NO production, but because of the compensatory action of H2O2, vasoregulation was not altered. In patient studies, H4B administration improved vasoregulation. The production of H2O2 may vary considerably between different types of small and large arteries. If H2O2 is also generated in human arteries in which a partial H4B deficiency exists, one wonders to what extent it acts on vasorelaxation in atherosclerotic arteries. The increased dimensions of the arteries in humans and the presence of multiple cell layers in the thickened intima, which are not involved in vasodilation 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 suppletments 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
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