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eNOS-overexpressing mice: too much NO makes the blood pressure low.

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Editorial

In this issue of the *Journal*, Ohashi and co-workers describe the generation of transgenic mice overexpressing the bovine endothelial cell nitric oxide synthase (eNOS) (1). The transgene, targeted to the endothelium using a preproendothelin-1 promoter, results in increased bovine eNOS mRNA and protein in the endothelium of the thoracic aorta, large pulmonary arteries and veins, and medium to small-sized coronary arteries.

Previous studies have demonstrated that in vivo inhibition of nitric oxide synthase by nonhydrolyzable analogues of L-arginine results in a dramatic increase in mean arterial blood pressure (2). Similarly, genetically engineered mice lacking the eNOS gene have impaired endothelium-dependent vasodilator responses to acetylcholine and are hypertensive (3). These two findings highlight the essential role of nitric oxide ($NO^{\bullet}$) in the maintenance of normal hemodynamics.

Given the above information, the outcome of overexpressing eNOS would seem obvious, i.e., a decrease in vascular tone leading to a reduction in blood pressure. However, before this study, it was unclear if increasing eNOS expression in the intact circulatory system would actually augment production of $NO^{\bullet}$ or affect systemic hemodynamics. eNOS protein activity is subject to remarkable posttranslational regulation, which affects its activity in both positive and negative manners (4). The most well-known control mechanism involves activation of calmodulin binding to the enzyme via increases in intracellular calcium levels. More recently, it has been shown that association with heat shock protein-90 markedly affects eNOS activity (5). Posttranslational palmitoylation, myristoylation, and phosphorylation target eNOS to plasmalemmal caveolae, where it undergoes protein–protein interactions with the structural protein, caveolin. It is now evident that caveolin inhibits eNOS activity and that a major signaling mechanism involves disassociation of eNOS from caveolin (4). Likewise, there is evidence that phosphorylation of eNOS may affect its activity and may activate the enzyme in a calcium-independent fashion (6). Finally, $NO^{\bullet}$ itself can inhibit the NO synthases in an autoinhibitory negative feedback fashion (7). Given these multiple levels of control of eNOS, it is entirely possible that increased expression of the enzyme might result in little or no increase in $NO^{\bullet}$ production in intact endothelial cells.

Nevertheless, the striking finding in this study by Ohashi et al. is that transgenic animals did have significantly lower blood pressures and higher plasma nitrite and nitrate levels (a measure of endogenous NO production) than their nontransgenic littermates. Thus, an important message to be learned is that overexpression of eNOS can indeed affect systemic hemodynamics by increasing endothelial cell $NO^{\bullet}$ production. This observation has implications for efforts to increase eNOS expression in humans using either physiological approaches (exercise training), pharmacological approaches (hormone replacement, antioxidants, HMG CoA reductase inhibitors), or gene therapy (8–10). A possible caveat is that the level of eNOS overexpression achieved by Ohashi was tremendous and may not be possible using these more conventional approaches.

Interestingly, although basal NO release was higher in aortas taken from transgenic mice, endothelium-dependent vascular relaxations and responses to exogenous $NO^{\bullet}$ donors were reduced. Although the mechanism for this remains unclear, it likely relates to a chronic downregulation, inhibition, or saturation of one or more or the components of the vascular smooth muscle targets of $NO^{\bullet}$, such as guanylate cyclase or the cGMP-dependent protein kinase. Further studies of these transgenic vessels after acute removal of the endothelium or administration of a nitric oxide synthase inhibitor would have been helpful, because ambient levels of NO are likely to have an immediate effect on the sensitivity of these downstream targets. It is possible that either one of these interventions would have immediately restored responses to exogenously administered $NO^{\bullet}$.

In cell culture and in vitro preparations, $NO^{\bullet}$ has been shown to have several putative antiatherosclerotic properties. These include potent inhibition of inflammatory molecules like VCAM-1 and MCP-1, inhibition of smooth muscle cell proliferation and inhibition of platelet aggregation (11). Despite these many observations in cell culture and in vitro, it has been difficult to demonstrate a beneficial effect of $NO^{\bullet}$ in preventing vascular disease in vivo. This is in large part because animals develop tolerance to exogenously administered NO donors rather rapidly, making long-term supplementation of NO impossible. In this regard, the eNOS-overexpressing mouse model developed in this study should be a very important research tool. For example, the role of endogenous $NO^{\bullet}$ in atherosclerosis can now readily be examined by crossing these mice with apo (E)–deficient mice or mice lacking the LDL receptor. Likewise, studies of vascular injury, neointimal development, and vascular remodeling in eNOS-overexpressing mice will be extremely revealing. The relative roles of NO produced by the endothelium versus adjacent parenchymal cells can also be examined using these animals. Together with similar experiments now being performed on the eNOS-deficient mouse, such research will provide a wealth of information regarding the role of endogenous NO in cardiovascular function and disease.

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