Nitric oxide (NO), the molecule of the year in 1995 and theme of the Nobel Prize in 1998, started its career as endothelium-derived relaxing factor (EDRF) almost 20 years ago. An unusual observation in the rabbit aorta, ie, the unexpected relaxation to acetylcholine only in preparations with endothelium, stimulated the scientific community because of its obvious physiological and clinical potential. The search for its identity left us with the surprising result that the answer was NO. This chemically unstable free radical and ancient mediator was fascinating not only because of its short half-life but also because it was the active component of all nitrovasodilators. Shortly thereafter, the enzyme nitric oxide synthase (NOS) was cloned and its substrate L-arginine identified. Thus, almost 100 years after the introduction of nitroglycerin in the treatment of angina pectoris, the endogenous nitrate was discovered, which, like its pharmacological counterpart, stimulated cGMP in vascular smooth muscle to cause vasodilatation.

Nitroglycerin is a very effective vasodilator of epicardial coronary arteries. Vasoconstriction of these vessels contributes importantly to ischemia and angina pectoris occurring during mental stress but also after cold exposure or exercise. True vasospasm can even cause angina at rest or trigger myocardial infarction. The effectiveness of nitroglycerin in many of these patients suggested that the vascular wall, and in particular the endothelium, might have a reduced capacity to release the endogenous nitrate NO. Indeed, many investigators showed that in patients with cardiovascular risk factors, such as hypertension, hypercholesteremia, smoking, or diabetes, endothelium-dependent relaxation is impaired (in fact, converted into a paradoxical vasoconstriction in response to many endothelium-dependent agonists) and the effects of NO inhibitors are reduced. Direct measurement of NO in human atherosclerotic plaques confirmed a markedly reduced expression of endothelial NOS (eNOS) and production of the endogenous nitrate in patients with atherosclerosis.

In this issue of Circulation, Nakayama et al report previously unknown mutations of the eNOS gene in patients with coronary vasospasm. The authors defined coronary spasm as an abnormal contraction of an epicardial coronary artery resulting in myocardial ischemia and not as an abnormal constrictor response to acetylcholine. The incidence of these 3 new and linked mutations in the 5′-flanking region of the eNOS gene was significantly greater in patients with coronary vasospasm than in control subjects. Importantly, using multiple logistic regression analysis in a rather large patient population, they found that the eNOS gene variant was the most predictive independent risk factor for coronary spasm. Most importantly, when they tested the functional consequences of these 3 mutations within the eNOS promoter using luciferase gene promoter assays, the T to C mutation proved to reduce promoter activity by approximately 50%. Clinically, it is of interest that patients who were homozygous for the mutant eNOS allele suffered from severe coronary vasospasm, and some of them even had experienced acute myocardial infarction in the absence of organic stenoses at angiography.

This is the first demonstration that mutations in the promoter of the eNOS gene are linked to a disease. It appears that in many patients with the mutant allele, an important endogenous vasodilator system, ie, the L-arginine–NO pathway, does not function properly, and hence, these patients are susceptible to vasoconstrictor stimuli. This is not surprising, because in vitro arterial segments without endothelium or those pretreated with an NO inhibitor such as Nω-nitro-L-arginine exhibit enhanced contractions to many vasoconstrictor agents. Similarly, infusion of NO inhibitors in vivo reduces blood flow and increases vasoconstrictor responses. Such exaggerated vasoconstrictor responses are also seen in patients with endothelial dysfunction. Another important mechanism of exaggerated vasoconstriction occurring under these conditions is related to endothelin. Endothelin production in the vascular wall is regulated by NO. Indeed, pharmacological inhibition of NO synthesis augments endothelin production in vitro. In vivo, Nω-nitro-L-arginine methyl ester increases vascular endothelin content and contributes to the increase in peripheral vascular resistance.

Increases in local vascular endothelin levels potentiate vasoconstrictor responses of the human coronary artery to serotonin and norepinephrine. Hence, the clinical consequences of reduced eNOS expression in carriers of the mutant gene, ie, coronary spasm, can be well explained by a reduced release of NO and enhanced vascular endothelin-1 production.

Obviously, such a mutation could have consequences not only in the coronary circulation, because eNOS expression would be impaired in all tissues in carriers of the mutant gene. In this context, it is of interest that patients with coronary...
spasm are known to suffer quite frequently from Raynaud’s disease, ocular spasm, and migraine. Although they did not investigate this, the findings of Nakayama et al support the concept that vasospasm is in principle a systemic disease with manifestations in different parts of the cardiovascular system.

The study was performed in a Japanese patient population, in which coronary vasospasm is known to occur more commonly than in many Western countries. In patients of Western populations, endothelial dysfunction and abnormal coronary vasomotion are seen primarily in the context of atherosclerotic coronary artery disease. Although differences between patients with true coronary spasm, particularly those seen in Japan, and the more common patient with coronary atherosclerosis have to be considered, it is of interest that in the vast majority of the initial patients of this study in whom the mutations were found, coronary spasm with ischemic ST-segment changes could be induced with acetylcholine, a feature shared by both forms of coronary artery disease. In early atherosclerosis, reduced NO production is related to increased oxidative stress as a result of the formation of superoxide radicals, which in turn inactivate NO and form the toxic end product peroxynitrate. The sources of these free radicals in endothelial cells are NAD(P)H oxidases and eNOS itself. Indeed, low intracellular levels of the essential cofactor tetrahydrobiopterin in hyperlipidemia are associated with dysfunction of eNOS and production of oxygen-derived free radicals. As judged from experimental models, eNOS expression at that stage appears to be maintained or even increased. At later stages, however, when atherosclerosis becomes clinically more relevant, eNOS expression is downregulated in patients with carotid atherosclerosis.14

Although many factors lead to endothelial dysfunction, it is noteworthy that in hyperlipidemia or hypertension, the degree of impairment of NO-dependent vasodilation varies considerably from patient to patient. Similarly, at the clinical levels, not all patients with cardiovascular risk factors get the disease, and conversely, some patients without known cardiovascular risk factors do get sick. Is it possible that mutations in the eNOS gene, particularly those that impair its function may make carriers prone to develop endothelial dysfunction and in turn coronary disease? Would genetic analysis of the eNOS gene therefore allow us to better characterize the actual risk of our patients beyond that already established by classic cardiovascular risk factors? Recently, another mutation of the eNOS gene in intron 4 was reported to be associated with the smoking-related risk for coronary artery disease.23 Other mutations will certainly be discovered in the near future.

Atherosclerotic coronary artery disease is a complex process triggered by many factors. In the Japanese population, environmental factors, in particular the high intake of ω-3 fatty acids, are at least in part responsible for the low incidence of coronary artery disease. On the other hand, coronary vasospasm without angiographically visible stenosis is quite common. It is conceivable that in the absence of hyperlipidemia, eNOS mutations are associated primarily with abnormal coronary vasomotion. In a population with higher fat intake, however, one would expect that carriers of a mutant gene would show more pronounced vascular alterations. Indeed, eNOS via activation of the transcription factor inhibitor IκB suppresses the expression of monocyte chemotactrant protein-1 and of several adhesion molecules. Oxidative stress, on the other hand, activates the transcription factor. These cellular events are important in early stages of atherosclerosis for the formation of fatty streaks. Furthermore, NO is an important inhibitor of platelet function and hence has an antithrombotic role.24 In a population such as the Japanese, who have a high intake of ω-3 fatty acids, platelets are more quiescent and produce less thrombogenic isoforms of thromboxane. Hence, alterations in the eNOS gene in Japanese may be less important for thrombus formation than in Western societies. Taken together, mutations in the eNOS gene may prove to be even more important for atherosclerotic coronary artery disease in Western societies than they are in Japanese patients as reported in this study.15

What would such findings mean for cardiovascular therapy? One obvious therapeutic measure in states with a reduced production of a given mediator would be its pharmacological substitution. Nitrate therapy would therefore seem to be the logical approach. However, recent findings showed that nitrates only in part mimic the effects of the endogenous nitrovasodilator system. Indeed, systemic application of nitrates has unfavorable hemodynamic effects, in particular activation of the sympathetic nervous system, not shared by the endogenous NO system, which is primarily activated locally within the vessel wall. Furthermore, nitrates stimulate vascular superoxide as well as endothelin production.26,27 These latter effects not only contribute to nitrate tolerance but also alter vascular function. Another approach would be to increase eNOS expression pharmacologically. Such effects have been attributed to statins28 and ACE inhibitors.29 Whether these pharmacological effects could also be obtained in carriers of eNOS mutations in the 5′-flanking region remains to be shown. It is possible that they may be ineffective because of the site of the mutation within the promoter of the eNOS gene. If so, it would make much more sense to substitute the mutated gene by a normal variant, ie, to use somatic gene therapy with a vector containing the normal eNOS gene to treat these patients.

Whatever the consequences of this study will be, it certainly will change our perception of the causes of endothelial dysfunction and cardiovascular disease and hopefully contribute to a better characterization of patients and to the development of more specific treatment modalities.

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References

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