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Endothelial-derived nitric oxide as a marker for healthy endothelium
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INTRODUCTION
In the past decade the importance of the vascular endothelium as a multifunctional regulator of vascular smooth muscle physiology and pathophysiology has been appreciated. Indeed, the endothelium responds to hemodynamic stimuli (pressure, shear stress and wall strain) and locally manufactured mediators (such as bradykinin, prostaglandins and angiotensin) and in turn can release factors that can influence the adhesion and aggregation of circulating cells to the endothelium and the tone of vascular smooth muscle. In many diseases, including cirrhosis, atherosclerosis or diabetes, endothelial dysfunction manifested as an impairment of nitric oxide (NO) production may be an early hallmark of disease and a treatable entity. In this chapter the importance of NO as a mediator of vascular function and potential mechanisms leading to endothelial dysfunction will be discussed.

REGULATION OF VASCULAR TONE BY ENDOGENOUS NO
Endothelial nitric oxide synthase (eNOS) is the NOS isoform responsible for producing the classical endothelium-derived relaxing factor as originally described by Furchgott and Zawadski. Evidence for the importance of eNOS-derived NO in the regulation of vascular tone is based on experiments in animals and in humans demonstrating that L-arginine-based inhibitors of NOS increase blood or perfusion pressure and vascular resistance and reduce blood flow in vivo and in vitro. More recently this has been unequivocally confirmed using mice with targeted disruption of the eNOS gene locus. eNOS knockout mice (−/−) are mildly hypertensive relative to wild-type littermate control mice (+/+ ) of the same generation. Importantly, the pressor effect of nitro-L-arginine, a NOS inhibitor, is attenuated in the −/− mice and endothelium-dependent relaxation in response to acetylcholine is abrogated in isolated vessels. This fundamental finding is direct “proof-
of-principle” for the major contribution of NO in vasomotor control in large blood vessels.

**PHYSIOLOGICAL ACTIVATION OF eNOS AND NO RELEASE**

Typically, endothelial cells release NO in response to autacoids that mobilize intracellular calcium such as thrombin, vascular endothelial growth factor (VEGF) or adenosine diphosphate (ADP). The proposed mechanism for eNOS activation is that the released calcium will bind to calmodulin (CaM) and the calcium/CaM complex will bind to the CaM site in the enzyme to promote NO synthesis. However, the most physiological agonist for NO release is fluid shear stress. Shear stress *in vitro*, or shear rate *in vivo*, is the tangential vector of force elicited by the flow of blood over the endothelial cell surface. Exposure of endothelial cells to shear stress results in a burst of NO release, followed by a sustained phase. *In vivo*, increasing shear rate due to high blood flow or vasoconstriction will cause flow-dependent dilations of certain vascular beds including the splanchnic circulation. Shear-induced NO release *in vitro* and flow-dependent vasodilation *in vivo* can be blocked with NOS inhibitors. Interestingly, shear-induced NO release appears to be “independent” of fluctuations of calcium since shear causes a rapid burst of calcium release that does not parallel the sustained release of NO; chelation of intracellular calcium does not influence the rate of NO production elicited by shear and CaM antagonists can block bradykinin-induced NO release but not shear-induced release. These data collectively suggest a fundamental difference in the signal transduction mechanisms for agonist versus shear- or growth factor-induced activation of eNOS.

**REGULATION OF NO PRODUCTION BY PROTEIN INTERACTIONS AND DISEASE**

eNOS is a membrane-associated NOS isoform that is modified by co-translational N-myristoylation at glycine 2 and post-translational cysteine palmitoylation at positions 15 and 26, and these fatty acids are important for its targeting in the Golgi region and plasmalemmal caveolae. The proper localization of eNOS is necessary for its interactions with other regulatory proteins (scaffolds, chaperones, kinases) that fine-tune the cycles of eNOS activation and inactivation.

The major negative regulatory protein for eNOS is caveolin-1. Caveolin-1 is the major coat protein of caveolae, and has several faces that may influence the biology of proteins that localize to cholesterol-rich plasmalemma caveolae. Indeed caveolin-1 is necessary for the biogenesis of caveolae through an unknown mechanism. In addition, caveolin-1 can serve as a cholesterol-binding protein and traffic cholesterol from the endoplasmic reticulum through the Golgi to the plasma membrane. Finally, caveolin has the capacity to directly interact with other intracellular proteins such as c-Src and H-Ras through amino acids 82–101, the putative scaffolding domain. Indeed, three groups independently demonstrated that eNOS could directly interact with caveolin-1 or caveolin-3. The primary binding region of caveolin-1...