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ScienceDirect
Free Radical Biology & Medicine 44 (2008) 1232–1245
www.elsevier.com/locate/freeradbiomed

Review Article

Regulation of smooth muscle by inducible nitric oxide synthase and NADPH oxidase in vascular proliferative diseases

Roman Ginnan, Benjamin J. Guikema, Katharine E. Halligan, Harold A. Singer, David Jourd'heuil *

Center for Cardiovascular Sciences, Albany Medical College, Albany, NY 12208, USA

Received 16 August 2007; revised 11 December 2007; accepted 11 December 2007
Available online 22 January 2008

Abstract

Inflammation plays a critical role in promoting smooth muscle migration and proliferation during vascular diseases such as postangioplasty restenosis and atherosclerosis. Another common feature of many vascular diseases is the contribution of reactive oxygen (ROS) and reactive nitrogen (RNS) species to vascular injury. Primary sources of ROS and RNS in smooth muscle are several isoforms of NADPH oxidase (Nox) and the cytokine-regulated inducible nitric oxide (NO) synthase (iNOS). One important example of the interaction between NO and ROS is the reaction of NO with superoxide to yield peroxynitrite, which may contribute to the pathogenesis of hypertension. In this review, we discuss the literature that supports an alternate possibility: Nox-derived ROS modulate NO bioavailability by altering the expression of iNOS. We highlight data showing coexpression of iNOS and Nox in vascular smooth muscle demonstrating the functional consequences of iNOS and Nox during vascular injury. We describe the relevant literature demonstrating that the mitogen-activated protein kinases are important modulators of proinflammatory cytokine-dependent expression of iNOS. A central hypothesis discussed is that ROS-dependent regulation of the serine/threonine kinase protein kinase Cδ is essential to understanding how Nox may regulate signaling pathways leading to iNOS expression. Overall, the integration of nonphagocytic NADPH oxidase with cytokine signaling in general and in vascular smooth muscle in particular is poorly understood and merits further investigation.

Keywords: Smooth muscle; Nitric oxide; iNOS; Superoxide; NADPH oxidase; Protein kinase C; MAP kinase; NF-κB; Free radicals

Contents

* Corresponding author. Fax: +1 518 262 8101.
E-mail address: jourdhd@mail.amc.edu (D. Jourd'heuil).

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doi:10.1016/j.freeradbiomed.2007.12.025

Introduction

Many vascular diseases such as atherosclerosis, postangioplasty restenosis, in-stent restenosis, and posttransplant coronary arteriopathy are characterized by intimal hyperplasia. Vascular smooth muscle (VSM) cells in the medial wall of blood vessels are normally quiescent and express a differentiated phenotype that serves to generate and maintain vascular tone [123]. In response to deendothelialization and increased exposure to cytokines and growth factors, VSM may dedifferentiate, migrate across the elastic lamina, and proliferate to form a neointimal layer and to secrete extracellular matrix components that form the bulk of the neointimal tissue [135]. Intimal hyperplasia results in vessel narrowing and clinically manifests itself in repeat adverse events such as myocardial infarction or repeat intervention. The molecular mechanisms underlying the VSM response to injury and the signaling pathways that control the integration of cytokines and growth factors to regulate VSM gene expression are not well understood. Overall, the complete etiology of intimal hyperplasia remains unknown.

Accumulating evidence points to vascular injury-induced production of reactive oxygen (ROS) and nitrogen (RNS) species as one important mechanism for the regulation of VSM migration and proliferation in the vascular wall. Recent advances have focused on the identification of nonphagocytic NADPH oxidases (Nox) that generate ROS and the realization that endogenously derived ROS regulate signaling pathways in all primary cellular components of the vascular wall. The contribution of nitric oxide synthase (NOS)-derived NO to vascular injury has been studied in greater detail and the convergence between ROS and RNS pathways has been focused on specific chemical interactions such as the reaction of NO with superoxide ($O_2^{\bullet-}$) [11] and lipid peroxyl radicals [130].

The alternative that ROS regulate NO production through regulation of inducible NOS (iNOS) expression has been poorly explored. Surely, the existing literature is pointing out the importance of both superoxide and hydrogen peroxide in regulating signaling pathways by influencing the activity of multiple kinases and phosphatases and modulating transcription factors. The nuclear transcription factor κB (NF-κB), a primary regulator of iNOS expression, is redox-regulated at multiple levels, including the binding of NF-κB to κB motifs [154], the regulation of the IκB kinase complex [88], and, potentially very upstream, the control of the endosomal targeting of cytokine receptors and Nox [101]. Direct evidence that the redox environment may regulate iNOS expression in vascular smooth muscle cells has been obtained: increased levels of the antioxidant enzyme catalase increase NF-κB activation and iNOS expression after cytokine stimulation of vascular smooth muscle cells [42,60]. These studies indicated that in this context cytokine-induced hydrogen peroxide production negatively regulates iNOS expression. The physiological significance and molecular mechanism underlying these observations are unknown, but would suggest that iNOS expression may be regulated by cytokine-mediated activation of Nox either at the level of or upstream of transcription factors. The primary purpose of this article is to review potential interactions between Nox signaling and iNOS expression in vascular smooth muscle. We summarize and contrast what is known regarding the distribution and roles of iNOS and Nox in the vasculature during injury. Given the complexity of iNOS transcriptional regulation, it would be impossible to cover all potentially relevant signaling pathways that might regulate iNOS expression in a redox-sensitive manner. Instead, we focus on one specific signaling molecule, protein kinase C, to exemplify some of the molecular mechanisms that may underlie the redox regulation of iNOS expression and cross talk between Nox and iNOS in vascular smooth muscle.

Inducible nitric oxide synthase: where, when, and how much?

Mammalian cells synthesize NO through the five-electron oxidation of one of the two-guanidino nitrogens of L-arginine [114]. A family of enzymes generically called nitric oxide synthase catalyzes this reaction from which three major classes have been described: neuronal NOS (nNOS, type I), iNOS (type II). and endothelial NOS (eNOS, type III). All three isoforms are expressed in the vasculature. The predominant isoform of NOS detectable in VSM in response to inflammatory cytokines is iNOS [45,84,170] and nNOS upregulation is induced in VSM by shear stress, hypoxia, and growth factors [116,124,159]. In the healthy vessel, the endothelium serves as the main source of NO production through eNOS activity to maintain vascular tone and regulate platelet aggregation and leukocyte adhesion [91,114,126]. Disruption of the endothelial layer and initial loss of eNOS is a hallmark of the development of atherosclerosis as well as restenosis. Traditionally, the upregulation of iNOS is perceived to compensate for the loss of a functional endothelium and eNOS during injury and atherosclerosis [61], although the presence of excess NO and ROS coincidentally may lead to additional tissue damage and dysfunction [84].

Early studies of the role of NOS and possible compensation for endothelial dysfunction focused on iNOS expression in plaque initiation and progression. The presence of iNOS mRNA and protein expression has been described in atherosclerotic human plaques in macrophages as well as endothelial and smooth muscle cells [106,162]. In support of a role for iNOS in promoting pathogenesis, iNOS expression was found in the majority of samples as early as the fatty streak stage and in all of the advanced stages of plaques coinciding with an increase in oxidized LDL and nitrated proteins [106]. The suggestion that iNOS expression and activity are correlated with lipid oxidation within the plaque is also supported by the observation that macrophage-derived iNOS colocalizes with oxidized lipid and protein derivatives found in atherosclerotic plaques, as well as nitrotyrosine in advanced atherosclerosis [28]. More recent studies support a dual role for iNOS in the development of the atherosclerotic plaque. In the ApoE$^{-/-}$ mouse model, iNOS is expressed in both macrophages and smooth muscle cells of the developing plaque, although smooth muscle cells are not present in early lesions [112]. In the advanced atherosclerotic plaques smooth muscle- and macrophage-derived iNOS may serve to continually promote a pathogenic environment by enhancing oxidative and nitrosative stress.