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Review Article

Pathogenetic role of eNOS uncoupling in cardiopulmonary disorders

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ABSTRACT

The homodimeric flavohemeprotein endothelial nitric oxide synthase (eNOS) oxidizes L-arginine to L-citrulline and nitric oxide (NO), which acutely vasodilates blood vessels and inhibits platelet aggregation. Chronically, eNOS has a major role in the regulation of blood pressure and prevention of atherosclerosis by decreasing leukocyte adhesion and smooth muscle proliferation. However, a disturbed vascular redox balance results in eNOS damage and uncoupling of oxygen activation from L-arginine conversion. Uncoupled eNOS monomerizes and generates reactive oxygen species (ROS) rather than NO. Indeed, eNOS uncoupling has been suggested as one of the main pathomechanisms in a broad range of cardiovascular and pulmonary disorders such as atherosclerosis, ventricular remodeling, and pulmonary hypertension. Therefore, modulating uncoupled eNOS, in particular eNOS-dependent ROS generation, is an attractive therapeutic approach to preventing and/or treating cardiopulmonary disorders, including protective effects during cardiothoracic surgery. This review provides a comprehensive overview of the pathogenetic role of uncoupled eNOS in both cardiovascular and pulmonary disorders. In addition, the related therapeutic possibilities such as supplementation with the eNOS substrate L-arginine, volatile NO, and direct NO donors as well as eNOS modulators such as the eNOS cofactor tetrahydrobiopterin and folic acid are discussed in detail.

Contents

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Introduction

It has been 30 years since Furchgott and Zawadski demonstrated that the endothelium produces a mysterious "endothelium-derived relaxing factor," which is responsible for vascular smooth muscle relaxation [1]. This was followed by the remarkable observation that this factor was the freely diffusible gas nitric oxide (NO) [2]. Today, NO is regarded as one of the body's most versatile molecules. It is a neurotransmitter, second messenger, inflammatory marker, and therapeutic agent that is generated by nitric oxide synthases (NOS). NOS catalyze the conversion of the amino acid L-arginine[3] and molecular oxygen to L-citrulline and NO, aided by cofactors. Three NOS isoforms have been identified, i.e., neuronal NOS (NOS-I), inducible NOS (NOS-II), and endothelial NOS (NOS-III; eNOS).

Endothelial NOS, as its name suggests, is mainly found in the endothelial lining of the blood vessels but also in the cardiomyocytes [4], airway epithelium [5], tubular cells of the kidney [6], and other organ systems. The cardiovascular system relies on eNOS for optimal function, most notably with NO as the principle mediator of flow-mediated dilation. In addition to blood pressure regulation, eNOS-derived NO is also responsible for inhibition of platelet aggregation, leukocyte adhesion, and smooth muscle cell proliferation (see Fig. 1 for the structure of eNOS) [7]. Consequently, functional impairment of this enzyme may result in endothelial dysfunction, leading to both pulmonary and systemic hypertension. Most actions of NO are mediated via the production of cGMP by guanylate cyclase, resulting in a decreased intracellular calcium (Ca^{2+}) concentration. Lower intracellular Ca^{2+} results in relaxation of the vascular smooth muscle layer and ultimately in vasodilatation and a decrease in blood pressure.

In this review, we discuss the molecular basis of eNOS uncoupling, the regulation of eNOS activity, its physiological functions in the cardiopulmonary system, and its role in the physiopathology of various cardiovascular and pulmonary diseases.

Molecular structure and function

eNOS is a homodimer that binds a number of different cofactors, which are required to convert L-arginine and O_2 to L-citrulline and NO. Each eNOS monomer has a bidomain structure. The N-terminus comprises the oxygenase domain and contains tetrahydrobiopterin (BH_4), heme iron, and L-arginine binding sites. L-Arginine and BH_4 promote enzyme dimerization and both act as a stabilizer of the active dimeric form [8,9]. The heme group is also essential for dimerization [10]. Further stabilization is generated by a zinc thiolate (ZnS_4) cluster formed by a zinc ion between two cysteine residues from each monomer. This cluster is responsible for the integrity of the BH_4 binding site [11]. The C-terminus is the reductase domain with binding sites for two flavins, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), and NADPH [12]. Electrons are produced by oxidation of NADPH to $NADP^+$ at the flavin domain of each monomer [12]. These electrons are then transferred, one at a

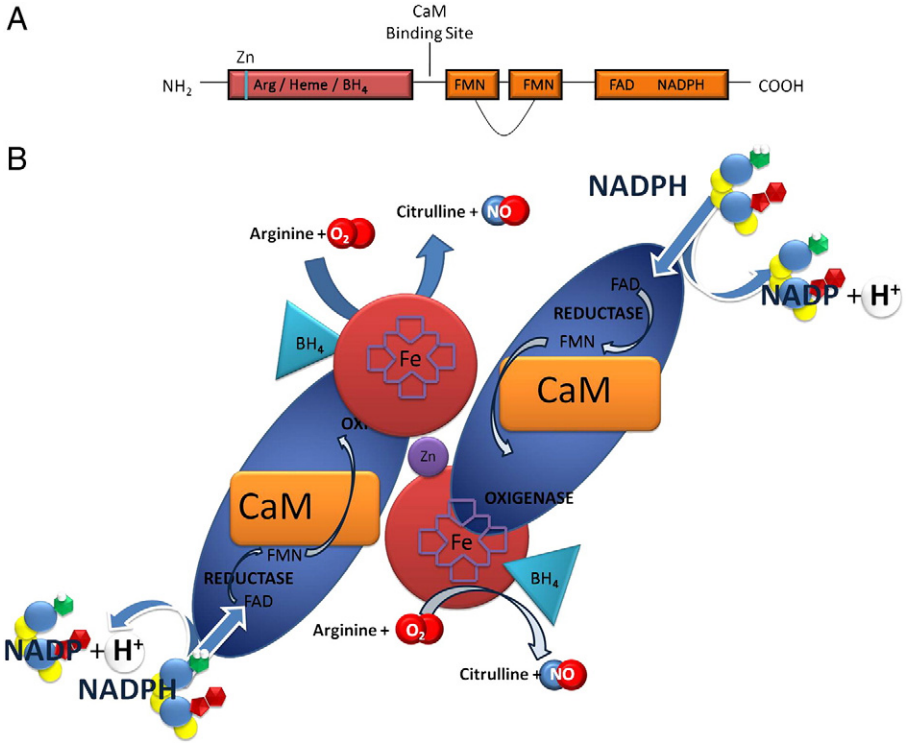

Fig. 1. Molecular structures of eNOS monomer and dimer. (A) Secondary structure of the eNOS monomer. The oxygenase (N-terminal) and reductase (C-terminal) domains are separated by a calmodulin (CaM) binding site. (B) Detail of the eNOS dimer. The Zn ion is responsible for connecting the monomers at the heme groups, which are resistant to dimerization. The electron transfer is visualized below, NADPH-oxidase donating an electron to be ultimately used to convert arginine and oxygen to the reaction products citrulline and nitric oxide. Tetrahydrobiopterin (BH_4) further stabilizes the dimer. Zn, zinc; Arg, arginine binding site; FMN, flavin mononucleotide; FAD, flavine adenine dinucleotide.