Coupling eNOS Uncoupling to the Innate Immune Response

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The endothelial isoform of nitric oxide synthase (eNOS) has been well recognized for its central role in conserving vascular homeostasis by inducing quiescence of endothelial cells and adjacent vessel wall structures. For example, eNOS-derived NO can alter protein function by S-nitrosylation of cysteine residues and, in endothelial cells, patterns of S-nitrosylated transcriptional regulators have been shown to interrupt inflammatory signaling and induce cell cycle arrest. Likewise, by competing with oxygen as an electron acceptor at complex IV, NO can limit energy expenditure through reducing oxidative phosphorylation during episodes of hypoxic challenge.

Yet eNOS has also been recognized as a source of non-nitrous reactive oxygen species that can elicit a coordinated proinflammatory response. This is related to the basic chemistry in the enzyme where NADPH-derived electrons flow from the reductase domain toward the oxygenase domain. With the cofactor calmodulin bound to the native homodimeric enzyme, and in the presence of tetrahydrobiopterin (BH4), these electrons react with oxygen and L-arginine and lead to the formation of L-citrulline and NO. However, if either one of these factors is (relatively) lacking, oxidation of oxygen occurs and the enzyme subsequently releases superoxide. This phenomenon, the so-called “uncoupling” of the eNOS, appears to be strongly conserved throughout evolution. It is therefore likely that this is not so much an anomaly of the enzyme but part of its physiological role in vascular homeostasis. As we recently proposed, activation of the innate immune system may probably lead to such uncoupling of the enzyme thus placing uncoupled eNOS in the center of the host defense response. For example, cytokines may induce arginase thus shutting L-arginine into the urea cycle, as well as induce the production of Asymmetrical Dimethyl Arginine (ADMA). This can lead to a relative deficit of substrate of the enzyme and uncoupling of eNOS.

In addition, immune activation has been associated with activation of NADPH oxidase both in phagocytes as well as endothelial cells. It has been suggested that the ensuing redox signaling may oxidize BH4 as well as reduce the regeneration of oxidized BH4. In either case, the oxidation substrate coupling may become disturbed and the eNOS enzyme may start to produce oxygen radicals. In support of this theory, supplementation with the cofactor BH4 has been shown to reduce the inflammatory response to organ transplantation. Further support for a role of eNOS in host defense was recently provided by a study that demonstrated increased bacterial invasion of the colon of eNOS knock-out mice in experimental colitis.

Although there is a clear rationale for eNOS uncoupling in host defense, the mechanism by which the innate immune system leads to endothelial cell activation is less clear. In the current study by Xu et al, the very interesting suggestion is made that hypochlorous acid (HOCl) could be a primary driver of the conversion of the quiescent endothelial state to one adapted for host defense. Using cultured endothelial cells as well as isolated mouse aortas, it is shown that HOCl may lead to uncoupling of the eNOS enzyme. This is of relevance as HOCl is produced by myeloperoxidase (MPO), the main constituent of azurophilic granules in neutrophils. HOCI is a potent bactericidal agent generated by the combined effects of MPO and the products of the NADPH-dependent oxidase released during the activation of phagocytes. After neutrophil degranulation, MPO gets deposited in and around the vascular endothelium and has been shown to modulate a proatherogenic vascular inflammatory response. Although the investigators did not investigate MPO itself, as MPO is unique in its ability to produce HOCI, the presented data are very intriguing and support a direct link between the neutrophilic innate immune response and eNOS uncoupling.

A second interesting aspect of the current study is that the authors demonstrate that HOCI leads to uncoupling of eNOS through normal signal transduction. Exposure of endothelial cells to HOCI leads to phosphorylation of the atypical protein kinase C-ζ, activation of NADPH-oxidase, and subsequently initiates the uncoupling response (Figure). This observation confirms a previous study that indicates a central role for the endothelial NADPH oxidase system in the modulation of eNOS uncoupling. However, the studies differ in the proposed molecular mechanism by which NADPH oxidase activation induces eNOS uncoupling. Calupsky et al exposed cultured bovine aortic endothelial cells to angiostatin II and observed an eNOS-dependent burst of superoxide production after 24 hours as a result of the depletion of BH4. They demonstrated that this loss of BH4 was caused by endothelial NADPH oxidase–derived H2O2 that led to a downregulation of dihydrofolate reductase the enzyme that normally catalyzes the regeneration of BH4 from its oxidized form dihydropterin. In the current study by Xu et al, eNOS uncoupling appears already within 2 hours after exposure of the endothelial cells to HOCI, and an alternative NADPH oxidase–dependent mechanism is proposed. They show that exposure of recombinant eNOS to HOCI oxidizes the zinc-thiolate center of eNOS and is associated with a loss of allosteric stability that leads to a dissociation of the homodimeric
enzyme into a monomeric form during SDS-PAGE analysis under reducing conditions. HOCl exposure to cultured endothelial cells and isolated mouse aortas also revealed a similar loss of allosteric stability secondary to NADPH oxidase activation. These intriguing observations raise several questions that remain unresolved. It is not clear whether the loss of the eNOS dimer structure under reducing conditions also reflects the loss of the dimer structure in vivo. Also, how specific is the observed effect specific for HOCl, or could other reactive oxygen or nitrogen species derived from innate immune cells also induce eNOS uncoupling? And finally, what mechanism underlies the HOCl-dependent activation of PKC-ζ?

Whatever the answers, the presented observations again support the hypothesis that uncoupling of eNOS is not merely an anomaly of nature but part of an integrated and controlled inflammatory signaling pathway that evolved for host defense.

Disclosures

None.

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