Coupled and Uncoupled NOS: Separate But Equal?
Uncoupled NOS in Endothelial Cells Is a Critical Pathway for Intracellular Signaling

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Is NOS Involved in Atherogenesis?

NO has been recognized as an antiatherogenic mediator at many points in the atherosclerotic process. Impaired aortic endothelium-dependent vasodilation caused by a dysfunctional NOS3–NO pathway is one of the early consequences associated with the major risk factors for the development of atherosclerosis such as hyperlipidemia, hypertension, diabetes, and smoking. However, in most animal models of atherosclerosis NOS3 protein expression is either unchanged or actually increased.9 This finding may be explained, in part, by evidence in the literature suggesting that NOS3 is uncoupled in atherosclerosis and hyperlipidemia to produce O2−. In contrast to NO, O2− has been demonstrated to be a proatherogenic mediator contributing to the development of atherosclerotic lesions.

Recent studies in genetically engineered and knock-out (KO) mice have implicated NOS3 in the progression of atherosclerosis. NOS3-KO mice fed a high-fat diet have a reduction in atherosclerotic lesion size compared with wild-type mice.10 NOS3 also contributes to the generation of oxidized low-density lipoprotein (oxLDL) and a proatherogenic phenotype. In addition, NOS3-overexpressing apoe-KO mice have significantly larger atherosclerotic lesions compared with control apoe-KO mice.2,9 In this latter study, additional experiments demonstrated that NOS3 was uncoupled, and supplementation with BH4 reduced the lesion size. These data implicate NOS3-derived O2− in the formation of atherosclerotic lesions and support the hypothesis that BH4 depletion results in the uncoupling of NOS3. The regulation of NOS3 enzymatic activity appears to play a major role in the balance of NO and O2− in the endothelial response during atherogenesis. Gharavi et al report that the mechanism by which oxidized phospholipids stimulate the subsequent upregulation of IL-8 is through the activation, and uncoupling, of NOS3 resulting in the production of ONOO−.

Oxidized phospholipids are localized in blood vessels at all stages of atherosclerosis and contribute to the progression of the disease. Berliner’s group has previously shown that oxidized 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine (Ox-PAPC) contributes to monocyte–endothelial cell adhesion and stimulates endothelial cells to synthesize chemotactic factors, including IL-8, via the activation of sterol regulatory element binding proteins (SREBPs).11,12 IL-8 is implicated in monocyte activation and endothelial chemotaxis during angiogenesis, and IL-8 levels have been shown to be elevated in atherosclerotic lesions.13 SREBPs are transcription factors that regulate cholesterol, fatty acid, triglyceride, and phospholipid synthesis.14 Recent work by Berliner’s laboratory demonstrated that Ox-PAPC treatment of endothelial

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cells results in depletion of caveolar cholesterol and activation of SREBP.
Therefore, in addition to stimulating IL-8 production, activation of SREBP by Ox-PAPC in endothelial cells promotes the atherosclerotic phenotype and this process is regulated by NOS3.

**Coupled NOS and Uncoupled NOS: Are There Two Pools of Enzyme Activity?**
Ghavari et al demonstrate that SREBP activation and IL-8 production by Ox-PAPC is NOS3-dependent, revealing a novel mechanism through which oxidized phospholipids mediate increases in the cytokine, IL-8. Ox-PAPC increases the phosphorylation of NOS3 on serine residue 1177 through the PI-3 kinase–Akt kinase pathway, independent of the c-Src kinase and cAMP-dependent protein kinase pathways. The authors also show that Ox-PAPC induces dephosphorylation of NOS3 on threonine residue 495. In unstimulated cultured endothelial cells, NOS3 is constitutively phosphorylated on threonine 495 and not phosphorylated on serine 1177. In response to stimulation (shear stress, VEGF, bradykinin, insulin, estrogen) NOS3 is rapidly phosphorylated on serine 1177 resulting in a two- to three-fold increase in NO production (for review, see reference 15). Constitutive phosphorylation on threonine 495 may interfere with the binding of calmodulin to NOS3 and is therefore associated with decreased enzymatic activity. Threonine 495 is dephosphorylated in response to stimuli that increase intracellular Ca2+ and results in an increase in NOS3 activity. Lin et al used a mutated T495A NOS3 that simulates the dephosphorylation and results in an increase in NOS3 activity. Lin et al also show that Ox-PAPC induces dephosphorylation of threonine 495 on NOS3 may act as a “switch” that uncouples NOS3 activity.

Ghavari et al demonstrate that Ox-PAPC stimulates NOS3 activity and that an NO donor is also able to mimic increases in IL-8 expression, supporting a role for NOS3-derived NO in this process. The authors further demonstrate that Ox-PAPC increases O2− production in endothelial cells that is blocked by a NOS inhibitor and that incubation with an ONOO− scavenger inhibited the Ox-PAPC–induced SREBP activation, supporting a role for NOS3-derived O2− as well. Thus, these data indicate that Ox-PAPC stimulates both NOS3 activity and the uncoupling of NOS3 to produce both NO and O2−, suggesting the possibility of two pools of active enzyme. The mechanism(s) of NOS3 uncoupling in this model system requires further investigation.

Fleming et al recently reported that oxLDL increases O2− production in endothelial cells that is blocked by a NOS inhibitor. This coincided with a decrease in phosphorylation at threonine 495 of NOS3 most likely attributable to oxLDL-induced decrease in protein kinase C activity. These authors found that NOS3 from the oxLDL-treated cells no longer bound calmodulin when stimulated, and that NOS3 was less prominently associated with the Golgi and plasma membranes resulting in cytosolic NOS3 distribution. Ox-PAPC is known to deplete caveolar cholesterol, which may result in a mislocalization of NOS3 contributing to uncoupling. Taken together these data suggest that NOS3 in endothelial cells may exist in two forms: coupled and uncoupled (see Figure).

The coupled enzyme is readily accessible to the “signalome” for activation and NO production, but the uncoupled enzyme is not. The uncoupled NOS3 enzyme may reside in the cytosol, whereas the coupled enzyme is associated with the membrane. Under pathological conditions, such as increased levels of oxidized phospholipids in the vasculature, an imbalance of coupled and uncoupled NOS3 would result in increased NOS3-derived O2− and further oxidation of phospholipids leading to the progression of atherosclerotic lesions.

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**References**


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**Diagram:**

A. Illustration of the appropriate “signalome” with membrane-associated NOS resulting in the phosphorylation and activation of NOS to produce NO.

B. Illustration of Ox-PAPC–induced cholesterol efflux resulting in a disruption of the NOS “signalome” and cytosolic localization of NOS.


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