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

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Endothelial dysfunction is seen early in the development of atherosclerosis, before overt vascular and structural changes. Nitric oxide (NO) is recognized as one of the major mediators of the maintenance of vascular homeostasis, and a decrease in NO bioavailability is associated with endothelial dysfunction. Endothelial NO synthase (eNOS; NOS3) catalyzes the formation of NO from L-arginine and O₂ in a reaction requiring Ca²⁺—calmodulin, FAD, FMN, NADPH, and tetrahydrobiopterin (BH₄). A decrease in NO bioavailability may be caused by: (1) a decrease in the expression or activity of NOS3, (2) uncoupling of NOS to produce superoxide (O₂⁻), or (3) degradation of NO by reacting with O₂⁻ from other enzymatic sources resulting in the formation of peroxynitrite (ONOO⁻). Physiologically, NOS3-derived NO inhibits leukocyte—endothelial cell adhesion, vascular smooth muscle proliferation and migration, and platelet aggregation to maintain the health of the vascular endothelium.

Under a number of pathological conditions, NOS3 enzymatic activity becomes uncoupled, resulting in the production of O₂⁻. NOS3-derived O₂⁻ has been shown to contribute to the development and progression of atherosclerosis and hypertension. In this issue of Circulation Research, Gharavi et al report that treatment of endothelial cells with oxidized phospholipids results in increased interleukin-8 (IL-8) production through the activation and uncoupling of NOS3. When NOS is uncoupled, electrons flowing from the redoxase domain to the heme are diverted to molecular oxygen instead of to L-arginine, resulting in the formation of O₂⁻. A number of potential mechanisms are responsible for uncoupling of NOS3, although the most consistent evidence exists for BH₄ deficiency. Also, NOS3 uncoupling has been shown to occur when: (1) there is a shortage of L-arginine or Hsp90, (2) NOS3 is dephosphorylated on threonine residue 495, or (3) NOS3 is redistributed to the cytosolic fraction of the cell. Gharavi et al suggest that phosphorylation of NOS3 at threonine 495 is involved in the uncoupling of NOS3 in their experimental model; however, the potential contribution of additional mechanisms was not examined.
cells results in depletion of caveolar cholesterol and activation of SREBPs. Therefore, in addition to stimulating IL-8 production, activation of SREBPs 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 used a mutated T495A NOS3 that simulates the dephosphorylation on 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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