Making Sense out of Oxygen Sensor

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Autoregulatory mechanisms that adjust the diameter of a vascular bed and the blood flow through it to the intrinsic parameters of tissue oxygen demand have become a cornerstone of vascular physiology. Although examples of hypoxia-induced vasoconstriction do exist (such as pulmonary microvasculature), most blood vessels respond to hypoxic or ischemic environment with vasodilation. Coronary arteries are not an exception to this rule; in fact, autoregulation in this vascular bed is of paramount importance. Increased myocardial demand for oxygen cannot be met by increasing oxygen extraction, because coronary arteriovenous difference is already high under normal conditions. Therefore, matching the supply depends almost entirely on the ability to increase coronary blood flow.

Although the occurrence of hypoxia- and ischemia-induced coronary vasodilation has been well established, the number of theories attempting to explain the phenomenon has multiplied. Proposed physiological models include (1) direct sensing of reduced PO2 by different cellular elements of the vascular wall; (2) production and release of vasodilatory metabolites by the oxygen-deprived myocardium; (3) changes in intracellular calcium or proton metabolism and distribution, or a rapidly developing deficiency in high-energy phosphates, which suppress the contractile apparatus of the smooth muscle cells; and (4) a shift in the affinity of hemoglobin for nitric oxide (NO). In this issue of Circulation Research, Shimizu et al provide further insight into the mechanisms of hypoxic vasorelaxation in porcine coronary arteries.

Previous studies by Daut et al, performed in guinea pigs, demonstrated the existence of glibenclamide-inhibitable coronary vasodilation in response to hypoxia or ischemia. On the basis of these findings, Shimizu et al launched a systematic investigation of the effect of K+ channel inhibitors on hypoxic vasorelaxation. The authors used inhibitors of several classes of K+ channels, specifically, Ca2+-dependent (tetraethylammonium, apamin, and charybdotoxin), voltage-dependent (4-aminopyridine), ATP-sensitive (glibenclamide), and inward rectifier (BaCl2), only to conclude that this mechanism is not operant in porcine coronary vessels. In addition, studies using calcium- and pH-sensitive fluorophores showed the lack of a clear correlation between changes in these parameters and hypoxic relaxation. Furthermore, hypoxic vasorelaxation did not appear to be related to the loss of endogenous phosphagens by porcine coronary arteries. Paradoxically, the force of smooth muscle contraction, but not ATP utilization, was increased under hypoxic conditions. To complete the picture, it is important to note that previous studies from the same laboratory using various bona fide pharmacological inhibitors have investigated the validity of several additional candidates for the role of oxygen sensor, including the Na+ pump, eicosanoids, hydrogen peroxide, superoxide, and protein kinases C and G, all of which have failed the selection process for an unambiguous factor responsible for hypoxic vasorelaxation. This exhaustive analysis is impressive, and the derived long list of trial-and-error eliminated candidates is palpably helpful, because it provides an ample opportunity to revisit the problem of oxygen sensing and vascular adaptation to oxygen deprivation.

Under the circumstances, it may be time to peer down the evolutionary road to oxygen sensing in bacteria and yeast in an attempt to discern the silhouettes of modern oxygen-sensing systems, such as NAD(P)H oxidase, heme proteins, and P-450 enzymes, as has been done in a recent article by Bunn and Poyton. Perhaps the most recent addition to this family of oxygen sensors or effectors, which I find intriguing, has been described in Drosophila. Hypoxia produced a series of rapid behavioral changes and induced cell cycle arrest, processes that were diminished by an inhibitor of NO synthase (NOS) and by inactivating mutations in the gene encoding for the cGMP-dependent protein kinase. Moreover, both short- and long-term responses to oxygen deprivation in Drosophila were dependent on NO. Although these examples of hypoxic adaptation are evolutionarily remote, I strongly believe that they may contribute fundamentally to our understanding of the problem of hypoxic relaxation of mammalian arteries. This optimism is based on the following circumstantial evidence.

Tissue responses to hypoxia are orchestrated by NO at different levels: (1) SNO-Hb[Fe(II)]O2 hemoglobin releases NO at decreased PO2; (2) glibenclamide-insensitive component of vasodilation could be due to the effect of low PO2 on the vascular endothelium to generate NO, as suggested by Daut et al; (3) NO can be displaced by carbon monoxide from the preexisting, probably heme-bound, cellular pool, where it is constantly replenished by the functioning endothelial NOS, thus exerting a rapid vasodilatory effect independent on the enzymatic machinery, as shown by Thorup et al; and (4) reduction in myocardial O2 consumption, so important in preconditioning, appears to be dependent on NO. All of these data point toward the possibility of NO mediation of adaptive responses to hypoxia.
NO-dependent hypothetical pathways of vascular autoregulation and adaptation to hypoxia. Cartoon depicts a portion of the plasma membrane, cytosolic compartment, and a mitochondrion in a vascular smooth muscle cell. A drop in oxygen tension results in the release of NO. This occurs from the preexisting pool of heme protein–bound NO as a result of decreased affinity for NO (similar to the mechanism described for hemoglobin by Stamler et al) or through the activation of mitochondrial NOS, a newly described calcium-sensitive constitutively expressed isoform of NOS. Increased mitochondrial NO levels result in the inhibition of cytochrome c oxidase, decline in mitochondrial membrane potential, and reduced proton-motive force for calcium uniporter. This then results in the inhibition of respiratory rate, reduced oxygen consumption, and generation of superoxide radicals. Reduced oxygen intermediates, in turn, suppress NO generation and provide a feedback regulatory loop to restore mitochondrial respiration. Under stress conditions, the operation of the mitochondrial NO system may play an important role in preventing mitochondrial calcium overload. Cytoplasmic NO liberation or generation also acts, directly or indirectly, on guanylate cyclase-cGMP–protein kinase G system and K⁺ or Ca²⁺ channels to elicit vasorelaxation and on HIF-1–mediated gene transcription (VEGF, inducible NOS, HO-1, and glycolytic enzymes). Note that this simplified model does not include an adjacent endothelial cell with abundant sources of NO production of its own together with the P450 and NAD(P)H oxidase systems, which are also engaged in orchestrating autoregulatory and adaptive responses to hypoxia. ROI indicates reduced oxygen intermediates; CcO, cytochrome c oxidase; MnSOD, Mn superoxide dismutase; Mt NOS, mitochondrial NO synthase; GC, guanylate cyclase; PKG, protein kinase G; VEGF, vascular endothelial growth factor; HIF-1, hypoxia-inducible factor-1; and HO-1, heme oxygenase-1.

Another breakthrough in our understanding of the oxygen-sensing and oxygen-signaling system came with the realization that immunogold–visualized endothelial NOS was detectable not only on the plasma membrane, as expected, but on the mitochondrial outer and inner membranes and cristae.

This enzyme was further characterized and found to represent a novel isoform, mitochondrial NOS, and NO was found to modulate the respiratory rate and ATP synthesis by inhibiting cytochrome c oxidase. The direct demonstration of the oxygen- and NO-dependent oxygen consumption in endothelial cells has been presented most recently. By imposing a continuous gradient of oxygen on endothelial cells, Clementi et al showed that the decreased oxygen consumption at low Po2 is mediated by NO inhibition of the cytochrome oxidase complex, or that “cell respiration was inhibited in parallel with the generation of NO” (page 1560). Collectively, these findings provide a more detailed view of the mitochondrial function, which may have a broad applicability to the problems of oxygen sensing and hypoxic vasorelaxation. The hypothetical schema of hypoxic regulation of O2 consumption and vasorelaxation is summarized in the Figure. This schema predicts that the drop in Po2 either stimulates NO production or liberates NO from heme-binding sites in the cytoplasm and mitochondrial matrix. Therefore, hypoxic relaxation is attributed to the cytoplasmic NO release, whereas hypoxia-induced reduction in oxygen consumption is attributed to the mitochondrial actions of NO. This schema gives credit to the existing strong evidence for heme-driven oxygen signaling. The possible role of yet another potent vasorelaxing substance, endothelium-derived hyperpolarizing factor, although shown to play a role in endothelium-dependent relaxation, requires definitive experimental proof in hypoxic vessels.

In summary, the emerging role of NOS, including the mitochondrial isoform, in orchestrating several vascular responses to hypoxia (eg, relaxation, reduced oxygen consumption, induction of HIF-1, vascular endothelial growth factor, inducible NOS, and glycolytic enzymes) is important. It can explain both the immediate and delayed responses to oxygen deprivation and unleashes an array of intermediary signals, all of which enormously complicate the molecular analysis of this vexed physiological system.

References


**Key Words:** oxygen sensor mitochondria nitric oxide heme proteins
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Circ Res. 2000;86:824-826
doi: 10.1161/01.RES.86.8.824

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