Hypoxic regulation of nitric oxide signaling in vascular smooth muscle

Thomas C. Resta
Vascular Physiology Group, Department of Cell Biology and Physiology, University of New Mexico Health Sciences Center, Albuquerque, New Mexico 87131-0001

The current article in focus by Mingone and colleagues (Ref. 16, see p. L296 in this issue) describes a novel permissive effect of hypoxia on cGMP-independent relaxation to nitric oxide (NO) in bovine pulmonary and coronary arteries. Interestingly, whereas NO-mediated vasorelaxation appeared to be largely a function of cGMP at ambient PO2, a cGMP-independent mechanism involving activation of the sarcoplasmic reticulum Ca2+ ATPase (SERCA) was revealed during hypoxic exposure. This study emphasizes the emerging complexity of NO signaling in vascular smooth muscle and raises important new questions regarding mechanisms by which PO2 regulates cGMP-independent actions of NO.

Both cGMP-dependent and -independent mechanisms of NO signaling have been implicated in vascular smooth muscle (1, 2, 4–8, 10, 13, 15, 16, 19, 20, 31, 32). It is well established that soluble guanylyl cyclase is an important target of NO in both the pulmonary and systemic circulations (8, 10, 13, 15, 19). Activation of soluble guanylyl cyclase by NO leads to increased cGMP synthesis and stimulation of protein kinase G (PKG) (8, 15, 19). PKG, in turn, elicits relaxation of vascular smooth muscle through a myriad of signaling pathways, leading to a decrease in the intracellular free Ca2+ concentration ([Ca2+]i) and desensitization of the contractile apparatus to Ca2+ (8, 15). Those pathways involving a decrease in [Ca2+]i are, for the most part, poorly understood. However, evidence exists for PKG-dependent activation of large-conductance Ca2+-activated K+ (BK) channels and associated membrane hyperpolarization, inhibition of L-type voltage-gated Ca2+ channels, stimulation of Ca2+-ATPases on both the plasma membrane and sarcoplasmic reticulum, inhibition of inositol trisphosphate receptors, and decreased inositol trisphosphate synthesis (8, 15). Those mechanisms involving a decrease in sensitivity of the contractile apparatus to Ca2+ in response to PKG activation are even less well defined but appear to be predominantly mediated by regulation of myosin light chain phosphorylation subsequent to activation of myosin light chain phosphatase (8, 15, 19, 21, 22).

In addition, NO signals through cGMP-independent mechanisms in many tissues by posttranslationally modify enzymatic activity (3, 11, 12, 18, 23–25, 29). Many cGMP-independent influences of NO in vascular smooth muscle appear to involve an increase in sarcolemmal K+ permeability. For example, NO has been reported to activate BK channels either directly (7) or indirectly via inhibition of 20-hydroxyecosatetraenoic (20-HETE) production (4, 5, 31) or increased calcium spark activity (20). Additional evidence supports a role for cGMP-independent activation of delayed rectifier K+ channels in pulmonary arterial smooth muscle cells (32). Other direct effects of NO include stimulation of SERCA in rabbit aortic smooth muscle, leading to increased Ca2+ reuptake by intracellular stores, a fall in [Ca2+]i, and consequent inhibition of store-operated Ca2+ influx (1, 2, 9). This latter mechanism of cGMP-independent activation of SERCA is consistent with the findings of Mingone et al. (16), which identify regulatory influences of hypoxia on this pathway.

Although the mechanism by which NO mediates cGMP-independent Ca2+ sequestration by the sarcoplasmic reticulum is not understood, it is possible that NO modulates SERCA activity by S-nitrosylation of the enzyme (25). It is also unclear how hypoxia functions to modify SERCA activity. Although current debate exists as to whether hypoxia increases the production of reactive oxygen species or instead leads to a more reduced cellular state in pulmonary vascular smooth muscle (14, 17, 26–30), one possible explanation for the permissive effect of hypoxia on NO-dependent stimulation of SERCA is via an alteration in the redox state of the pump. In agreement with this hypothesis, Adachi et al. (2) have demonstrated that impaired NO-dependent relaxation and SERCA activity in aortas from hypercholesterolemic rabbits are prevented by long-term administration of the antioxidant t-butylhydroxytoluene, suggesting that increased oxidative stress associated with hypercholesterolemia impairs SERCA function. A more reduced state imposed by hypoxia may, therefore, enhance refilling of intracellular Ca2+ stores through disinhibition of SERCA. Whether oxidative stress impairs SERCA activity by preventing S-nitrosylation of the pump remains to be investigated. However, a recent report by Sun et al. (24) has demonstrated a similar inhibitory effect of increasing PO2 on both NO-dependent activation and S-nitrosylation of the skeletal muscle ryanodine receptor. Whereas stimulation of channel activity by NO is inhibited at ambient PO2 (~150 mmHg), an

Address for reprint requests and other correspondence: T. C. Resta, Vascular Physiology Group, Dept. of Cell Biology and Physiology, Univ. of New Mexico Health Sciences Center, MSC08 4750, 1 Univ. of New Mexico, Albuquerque, NM 87131-0001 (E-mail: tresta@salud.unm.edu).
apparently lower oxidative stress associated with a more physiological tissue PO₂ (≈10 mmHg) permits S-nitrosylation at a specific cysteine residue (Cys-3635) via an allosteric mechanism and subsequent channel activation. It is noteworthy that this lower PO₂ is similar to the oxygen tension used as a hypoxic stimulus (8–10 mmHg) by Mingone et al. (16), and thus an analogous mechanism may account for the observed cGMP-independent stimulation of SERCA by NO under hypoxic conditions. In contrast to potential influences of hypoxia on the redox state and allosteric regulation of the target enzyme (e.g., SERCA), it is alternatively possible that hypoxia limits NO bioinactivation by oxygen-derived free radicals, thus facilitating S-nitrosylation reactions through increases in the concentration of NO within relevant microvascular domains.

In conclusion, the observations of Mingone and colleagues (16) will likely provide the basis for intriguing new avenues of investigation to understand mechanisms of hypoxic modulation of NO signal transduction in vascular smooth muscle. Challenges of future studies include defining the potential role of S-nitrosylation of SERCA in regulating pump activity as well as the mechanism by which hypoxia facilitates cGMP-independent stimulation of SERCA by NO. The concept of hypoxia as a redox effector of second messenger signaling has potentially broad physiological and pathophysiological implications for not only NO regulation of vascular, immunological, and neural function, but for hypoxic modulation of a diverse spectrum of intracellular signaling pathways.

REFERENCES


9. Cohen RA, Weisbrod RM, Geremie M, Yaghoubi M, Bierl C, and Bolotina VM. Mechanism of nitric oxide-induced vasodila-


27. Waypa GB, Marks JD, Mack MM, Boriboun C, Mungai PT, and Schumacker PT. Mitochondrial reactive oxygen species


