Transnitrosation Signals Oxyhemoglobin Desaturation

Nadzeya Marozkina, Benjamin Gaston, Allan Doctor

Erythrocytes dilate peripheral blood vessels as a function of oxyhemoglobin desaturation. This effect increases regional blood flow to hypoxic tissues. The mechanisms underlying the peripheral vasodilatory effects of desaturating erythrocytes are incompletely understood but do not involve activation of local, endothelial NO synthase (eNOS). Indeed, eNOS-derived NO itself primarily relaxes large vessels and does that primarily only in the absence of blood.

In this issue of Circulation Research, Diesen et al confirm that thiols carrying a nitrosonium (NO⁺) equivalent signal cyclic GMP-dependent vascular smooth muscle relaxation during erythrocytic oxyhemoglobin desaturation. These data support paradigm-changing work demonstrating that nitrogen oxides are transported by circulating erythrocytes to signal oxyhemoglobin desaturation through serial NO/NO⁺ thiol equilibria and transfer reactions (transnitrosation) and that these reactions normally take place at sites remote from NOS activity. These new data show clearly that this signaling is independent of local NO activity, of cyclooxygenase, of ATP, and of the effects of hypoxia itself on vascular smooth muscle.

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endothelium both acutely through S-nitrosylation of metallothionein and chronically through upregulation of hypoxia and S-nitrosothiol-dependent genes that cause vascular remodeling. Indeed, this construct suggests a unifying hypothesis underlying pulmonary arterial hypertension. Specifically, pulmonary arterial hypertension can be caused by (1) chronic systemic hypoxia (causing excessive S-nitrosothiol-mediated NO/NO transfer to thiols in erythrocytes returning to the right heart and pulmonary artery); (2) chronically increased blood flow to the pulmonary artery (increasing the numbers of S-nitrosothiol-bearing erythrocytes to pass through the pulmonary vasculature endothelium, particularly in the context of polycythemia); (3) chronic inflammation (increasing the number of S-nitrosothiols in red cells); and (4) chronic N-acetylcysteine administration (excessively transferring NO/NO to the pulmonary vascular endothelium).

In normal human physiology, endogenous NO does not appear to signal erythrocyte deoxygenation. Diesen et al show that physiologically relevant concentrations of NO have no effect on vascular smooth muscle tone in hypoxia. Support for the idea that NO reductase function of Hb would serve physiological function has rested in 3 legs. Firstly, it is known that pharmacological (high micromolar, 2 to 3 orders of magnitude higher than physiological) concentrations of NO, injected into an artery, will cause weak vasodilatation. This mechanism likely involves oxidation of Fe(II) Hb to Fe(III), with the formation of Fe(III)-NO species in equilibrium with an Fe(II)-NO that is capable of modifying thiols through transnitrosation. Whereas this mechanism may be operative in the formation of S-nitroso-Hb and in response to high (pharmacological) concentrations of NO, it is not operative during normal hypoxic signaling by which erythrocytes caused cGMP-dependent vascular smooth muscle responses: neither the concentrations nor the kinetics are in the range of concentrations or blood flow rates in vivo.

Secondly, it has been argued that Hb micropopulations respond at the Hb P50 to form and release NO from NO, which escapes autocapture from other heme groups. However, the physiological response under study, hypoxic vasodilatation does not demonstrate a P50 threshold: it is a graded effect that increases at decreasing level of oxyhemoglobin saturation. Moreover, NO does not diffuse away from Fe(II) heme-containing erythrocytes in any physiologically relevant concentration.

Finally, high nanomolar concentrations of NO (in the presence of deoxyhemoglobin) were once proposed to cause vascular smooth muscle relaxation. Five years later, these data still await confirmation at these concentrations. Diesen et al did not observe the same effect. Note in this regard that some of the effects reported to be NO/N radical-mediated may reflect inorganic NO protonation at low pH.

Confusion regarding erythrocytic NO metabolism has arisen because of the use of iodine-based assays to measure S-nitrosothiols. These assays may misidentify nitrogen oxide species and require sample handling that alters Hb allostery before measurement. Indeed, what has been reported as erythrocytic NO using an iodine-based assay was lost with protein precipitation.

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**Figure.** Transnitrosation signals Hb desaturation. Erythrocytes are endogenously preloaded with NO/nitrosonium in the form of S-nitrosohemoglobin. Oxyhemoglobin desaturation exposes the S-nitrosothiol bond of S-nitrosohemoglobin to transnitrosation reactions with endogenous and exogenous low-mass thiols (RSN) and with erythrocytic protein thiols (eg, anion exchange protein (AE1)) during R-to-T conformational change. Low-mass S-nitrosothiols (R-SNO) can enter endothelial cells, where they S-nitrosylate specific protein targets, including gene regulatory proteins involved in vascular remodeling and in physiological regulation. Also, through subsequent S-nitrosylation reactions and/or homolytic cleavage to NO, they can cause cGMP-dependent smooth muscle relaxation.
More work remains regarding the effects of S-nitrosothiol depletion and transnitrosation-based augmentation of vasculart smooth muscle relaxant effects in vivo, at more physiological hematocrit values and using direct blood flow measurements in hypoxic resistance arterioles. Additionally, virtually all the relevant studies regarding hypoxic signaling remain to be done in the βCys93-deficient mouse.

In conclusion, Diesen et al confirm the S-nitrosothiol-mediated mechanism by which cGMP-dependent vascular smooth muscle relaxation may be signaled by erythrocyte deoxygenation. Moreover, they demonstrate that transnitrosation from an erythrocyte thiol to a target thiol augments relaxation, consistent with a paradigm by which S-nitrosothiols can cause acute vascular effects, as well as vascular remodeling in the face of chronic, excessive S-nitrosothiol “dumping” in the pulmonary arterial bed. Their data thus confirm the novel paradigm in which it is not O2 tension per se that is sensed in hypoxic vascular beds but rather oxyhemoglobin desaturation. This concept has been slow to gain acceptance, primarily because it requires an appreciation that the effector molecules in physiology and in disease pathophysiology.

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