NIH Public Access
Author Manuscript
Circ Res. Author manuscript; available in PMC 2009 September 5.

Published in final edited form as:
Circ Res. 2008 August 29; 103(5): 441–443. doi:10.1161/CIRCRESAHA.108.183442.

Transnitrosation signals oxyhemoglobin desaturation

Nadzeya Marozkina[2], **Benjamin Gaston**[2], and **Allan Doctor**[1]

[1]Department of Pediatric Critical Care, Washington University School of Medicine, St. Louis, MO 63110

[2]Division of Pediatric Respiratory Medicine, University of Virginia School of Medicine, Charlottesville, VA 22098

Keywords

S-nitrosylation; Hypoxia; Blood flow

Erythrocytes dilate peripheral blood vessels as a function of oxyhemoglobin desaturation.[1] The mechanisms underlying this effect are incompletely understood, but do not involve activation of local, endothelial nitric oxide synthase (NOS). In this month's Circulation Research, Diesen and coworkers confirm that thiols carrying a nitrosonium (NO^+) equivalent signal cyclic GMP-dependent vascular smooth muscle relaxation during erythrocytic oxyhemoglobin desaturation.[2] 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.[1-3] These new data show clearly that this signaling is independent of local NOS activity, of cyclooxygenase, of ATP, and of the effects of hypoxia itself on vascular smooth muscle.

Erythrocytes are endogenously "pre-loaded" with nitrogen oxides for delivery to vessels in conditions of oxyhemoglobin desaturation. [1-3] This signaling links delivery of erythrocytic NO/NO^+ groups to oxyhemoglobin desaturation, permitting augmented blood flow to hypoxic tissue. Three mechanisms have been proposed by which transitions in hemoglobin (Hb) conformation may result in nitrogen oxide transfer to blood vessels. 1. In the originally proposed mechanism, Hb deoxygenation (R to T transition) permits transnitrosation from Hb β chain cysteine 93 (βCys93) to erythrocytic carrier thiols (Figure).[1-3] This concept is supported by the data of Diesen and coworkers.[2] 2. At one time, NO radical was proposed to diffuse away from the Fe (II) heme iron in T state hemoglobin. Though there was an initial enthusiasm for this construct as an NO radical-based alternative to nitrosothiol-based NO^+ transfer, it is essentially impossible kinetically; it is now acknowledged to be irrelevant to physiology.[1, 6, 7] 3. More recently, it has been proposed that deoxyhemoglobin serves as a NO_2^- reductase, forming an NO radical that, again, escapes autocapture by Fe(II) heme to diffuse into—and activate guanylate cyclase in—smooth muscle cells.[8] This mechanism is also essentially impossible kinetically but is still referenced. The data of Diesen and coworkers argue against this construct, which is discussed further below.

It had been suggested that NO^+ transfer from βCys93 in deoxygenating hemoglobin might not be relevant to physiology because mice genetically engineered to be deficient in the βCys93

Corresponding Author: Benjamin Gaston, MD Box 800386 University of Virginia School of Medicine Charlottesville, VA 22908 Phone: 434-924-1820 Fax: 434-924-8388 Email: bmg3g@virginia.edu.

Disclosures: Dr. Gaston is a consultant for Galleon Pharmaceuticals, Inc.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

were once rumored not to have had abnormalities in hypoxic responses. However, it turns out that none of the physiological responses to Hb desaturation demonstrated to hinge on transnitrosation have actually been tested in the βCys93-deficient mouse: Specifically, there are three such responses; none of the three were studied. These are: 1) pulmonary vascular remodeling in chronic hypoxemia;[5] 2) altered blood flow in vascular beds in response to altered oxyHb saturation [1,9,10] ; and 3) augmentated minute ventilation in response to hypoxia.[11] Even assuming that humanized Hb knocked in to these mice behaves identically to murine Hb—which it likely does not—they were only studied with regard to overall blood pressure responses[8]. Blood pressure is not a surrogate for regional blood flow distribution. Further, the work by Diesen and coworkers provides insight into the negative vascular ring study using erythrocytes from these mice. Specifically, βCys93 SNO bonds can be rapidly depleted in wild-type red cells [2, 12, 13], ablating their ability to relax vascular smooth muscle with Hb desaturation. The pulmonary vascular ring responses in the studies of the βCys93 deficient mouse were minimal: either the control RBCs were S-Nitrosothiol depleted - rendering them functionally indistinguishable from the RBCs of βCys93-deficient RBCs — or the vascular rings were essentially unresponsive.

Diesen and coworkers confirm previous studies that transnitrosation from erythrocytic S-nitrosothiols forms low-mass S-nitrosothiols that can signal vascular effects[4,6-11] (Figure). Chronic *in vivo* transnitrosation from hypoxic erythrocytes to N-acetyl-cysteine - forming S-nitroso-N-acetyl cysteine - causes pulmonary vascular remodeling through upregulation of hypoxia- and S-nitrosothiol-sensitive genes in the pulmonary endothelium.[5] Diesen and coworkers now show that erythrocytic transnitrosation to N-acetyl-cysteine and other low-mass thiols potentiates acute vascular smooth muscle effects of erythrocytic S-nitrosothiols in the context of hemoglobin deoxygenation.

We thus see a picture emerge of elegant regulation: NO^+ transfer from deoxyhemoglobin thiols to erythrocytic low-mass and membrane[14] thiols permits NO/NO^+ transfer to target proteins in the vasculature. Thus, acute hypoxic effects are actually signaled by oxyhemoglobin desaturation rather than low pO_2. These acute effects include increased minute ventilation and cyclic GMP-dependent vascular smooth muscle relaxation.[1, 2, 11] However, excessive NO^+ transfer reactions can signal a counterregulatory, protective effects in the endothelium both acutely links through S-nitrosylation of metallothionein[15] and chronically, through upregulation of hypoxia and S-nitrosothiol-dependent genes that cause vascular remodeling.[5] 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^+ transfer to thiols in erythrocytes returning to the right heart and pulmonary artery); 2) chronically increased blood flow to the pulmonary artery (with increased numbers of S-nitrosothiol-bearing erythrocytes to pass through the pulmonary vasculature endothelium, particularly in the context of polycythemia); chronic inflammation (increasing the number of S-nitrosothiols in red cells); and chronic NAC administration (excessively transferring NO^+ to the pulmonary vascular endothelium).[5, 16]

In physiology, nitrite does not appear to signal erythrocyte deoxygenation. Diesen and coworkers show that physiologically relevant concentrations of nitrite have no effect on vascular smooth muscle tone in hypoxia.[2] Support for the idea that a NO_2- reductase function of hemoglobin would serve physiologic function has rested in three legs. First, it is known that pharmacological (high μM, 2 -3 orders of magnitude higher than physiological) concentrations of NO_2-, 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.[17, 18] Whereas this mechanism may be operative in the formation of S-nitroso-Hb and in

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

response to high (pharmacologic) concentrations of nitrite, 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* 1,[19].

Secondly, it has been argued that Hb micropopulations respond at the Hb P_{50} to form and release NO, which escapes autocapture from other heme groups. However, the physiologic response under study, hypoxic vasodilatation, does not demonstrate a P_{50} threshold: it is a graded effect that increases at decreasing level of oxyhemoglobin saturation.[20] Moreover, NO does not diffuse away from Fe(II) heme-containing erythrocytes in any physiologically relevant concentration.[19]

Finally, high nM concentrations of nitrite—in the presence of deoxyhemoglobin—were once proposed to cause vascular smooth muscle relaxation.[21] Five years later, this these data still await confirmation at these concentrations; Diesen and coworkers, did not observe the same effect. Note in this regard that some of the effects reported to be nitrite/NO radical-mediated may reflect inorganic nitrite protonation at low pH.[22]

Confusion regarding erythrocytic nitric oxide metabolism has arisen because of the use of iodine-based assays to measure S-nitrosothiols. These assays mis-identify all nitrogen oxide species and require sample handling that alters hemoglobin allostery prior to measurement.[6, 23] Though they have been "validated" against themselves under artificial conditions, they fail reliably to measure any erythrocytic nitrogen oxide species . Indeed, what has been reported as erythrocytic nitrite using an iodine based assay actually turns out to be lost with protein precipitation.[24]

More work remains regarding the effects of S-nitrosothiol depletion and transnitrosation-based augmentation of vascular 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 and coworkers confirm athe 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 O_2 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 are S-nitrosothiol/NO^+ donors, rather than NO radical. However, it provides an elegant mechanism to explain a broad range of previously obscure effects in physiology and in disease pathophysiology.

Acknowledgments

Sources of Funding: Benjamin Gaston and Nadzeya Marozkina: 3RO1 HL59337; SARP 2RO1, LH69170; Allan Doctor: 1K08GM069977

References

1. Singel DJ, Stamler JS. Chemical physiology of blood flow regulation by red blood cells: the role of nitric oxide and S-nitrosohemoglobin. Ann Rev Physiol 2005;67:99–145. [PubMed: 15709954]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

2. Diesen KL, Hess DT, Stamler JS. Hypoxic vasodilatation by red blood cells: evidence for an S-nitrosothiol-based signal. Circ Res 2008;103:XXX–XXX.

3. Jia L, Bonaventura C, Bonaventura J, Stamler JS. S-nitrosohaemoglobin: a dynamic activity of blood involved in vascular control. Nature 1996;380:221–226. [PubMed: 8637569]

4. Doctor A, Platt R, Sheram ML, Eischeid A, McMahon T, Maxey T, Doherty J, Axelrod M, Kline J, Gurka M, Gow A, Gaston B. Hemoglobin conformation couples S-nitrosothiol content in erythrocytes to oxygen gradients. Proc Natl Acad Sci USA 2005;102:5709–5714. [PubMed: 15824313]

5. Palmer L, Doctor A, Chhabra P, Sheram ML, Laubach V, Karlinsey MZ, Forbes M, Macdonald T, Gaston B. S-Nitrosothiols signal hypoxia-mimetic vascular pathology. J Clin Invest 2007;117:2592–2601. [PubMed: 17786245]

6. Gaston B, Doctor A, Singel D, Stamler JS. S-Nitrosothiol signaling in respiratory biology. Am J Respir Crit Care Med 2006;173:1186–93. [PubMed: 16528016]

7. Jeffers A, Xu X, Huang K, Cho M, Hogg N, Patel RP, Kim-Shapiro DB. Hemoglobin mediated nitrite activation of soluble guanylyl cyclase. Compar Biochem Physiol 2005;142:130–135.

8. Isbell TS, Sun CW, Wu LC, Teng X, Vitturi DA, Branch BG, Kevil CG, Peng N, Wyss JM, Ambalavanan N, Schwiebert L, Ren J, Pawlik KM, Renfrow MB, Patel RP, Townes TM. SNO-hemoglobin is not essential for red blood cell-dependent hypoxic vasodilation. Nature Med 2008;14:773–7. [PubMed: 18516054]

9. Sonveaux P, Kaz AM, Snyder SA, Richardson RA, Cardenas-Navia LI, Braun RD, Pawloski JR, Tozer GM, Bonaventura J, McMahon TJ, Stamler JS, Dewhirst MW. Oxygen regulation of tumor perfusion by S-nitrosohemoglobin reveals a pressor activity of nitric oxide. Cir Res 2005;96:1119–1126.

10. McMahon TJ, Ahearn GS, Moya MP, Gow AJ, Huang YC, Luchsinger BP, Nudelman R, Yan Y, Krichman AD, Bashore TM, Califf RM, Singel DJ, Piantadosi CA, Tapson VF, Stamler JS. A nitric oxide processing defect of red blood cells created by hypoxia: deficiency of S-nitrosohemoglobin in pulmonary hypertension. Proc Natl Acad Sci USA 2005;102:14801–6. [PubMed: 16203976]

11. Lipton A, Johnson M, Macdonald T, Lieberman M, Gozal D, Gaston B. S-Nitrosothiols signal the ventilatory response to hypoxia. Nature 2001;413:171–174. [PubMed: 11557982]

12. Bennett-Guerrero E, Veldman TH, Doctor A, Telen MJ, Ortel TL, Reid TS, Mulherin MA, Zhu H, Buck RD, Califf RM, McMahon TJ. Evolution of adverse changes in stored RBCs. Proc Natl Acad Sci USA 2007;104:17063–8. [PubMed: 17940021]

13. Reynolds JD, Ahearn GS, Angelo M, Zhang J, Cobb F, Stamler JS. S-nitrosohemoglobin deficiency: a mechanism for loss of physiological activity in banked blood. Proc Natl Acad Sci USA 2007;104:17058–62. [PubMed: 17940022]

14. Pawloski JR, Hess DT, Stamler JS. Export by red blood cells of nitric oxide bioactivity. Nature 2001;409:622–626. [PubMed: 11214321]

15. Bernal PJ, Leelavanichkul K, Bauer E, Cao R, Wilson A, Wasserloos KJ, Watkins SC, Pitt BR, Croix CM. Nitric oxide-mediated zinc release contributes to hypoxic regulation of pulmonary vascular tone. Circ Res 2008;102:1575–83. [PubMed: 18483408]

16. Marsden PA. Low-molecular-weight S-nitrosothiols and blood vessel injury. J. Clin. Invest 2007;117:2377–80. [PubMed: 17786231]

17. Angelo M, Singel DJ, Stamler JS. An S-nitrosothiol (SNO) synthase function of hemoglobin that utilizes nitrite as a substrate. Proc Natl Acad Sci USA 2006;103:8366–8371. [PubMed: 16717191]

18. Nagababu E, Ramasamy S, Rifkind JM. S-nitrosohemoglobin: a mechanism for its formation in conjunction with nitrite reduction by deoxyhemoglobin. Nitric Oxide 2006;15:20–29. [PubMed: 16545588]

19. Jeffers A, Xu X, Huang KT, Cho M, Hogg N, Patel RP, Kim-Shapiro DB. Hemoglobin mediated nitrite activation of soluble guanylyl cyclase. Compar Biochem Physiolt A 2005;142:130–5.

20. Ross JM, Fairchild HM, Weldy J, Guyton AC. Autoregulation of blood flow by oxygen lack. Am J Physiol 1962;202:21–24. [PubMed: 14494191]

21. Cosby K, Partovi KS, Crawford JH, Patel RP, Reiter CD, Martyr S, Yang BK, Waclawiw MA, Zalos G, Xu X, Huang KT, Shields H, Kim-Shapiro DB, Schechter AN, Cannon RO 3rd, Gladwin MT. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. Nature Med 2003;9:1498–505. [PubMed: 14595407]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

22. Gaston B, Kelly R, Urban P, Liu L, Henderson EM, Doctor A, Teague WG, Fitzpatrick A, Erzurum S, Hunt JF. Buffering airway acid decreases exhaled nitric oxide in asthma. J Allerg Clin Immunol 2006;118:817–22.

23. Hausladen A, Rafikov R, Angelo M, Singel DJ, Nudler E, Stamler JS. Assessment of nitric oxide signals by triiodide chemiluminescence. Proc Natl Acad Sc USA 2007;104:2157–62. [PubMed: 17287342]

24. Rogers SC, Khalatbari A, Datta BN, Ellery S, Paul V, Frenneaux MP, James PE. NO metabolite flux across the human coronary circulation. Cardiovasc Res 2007;75:434–41. [PubMed: 17512506]

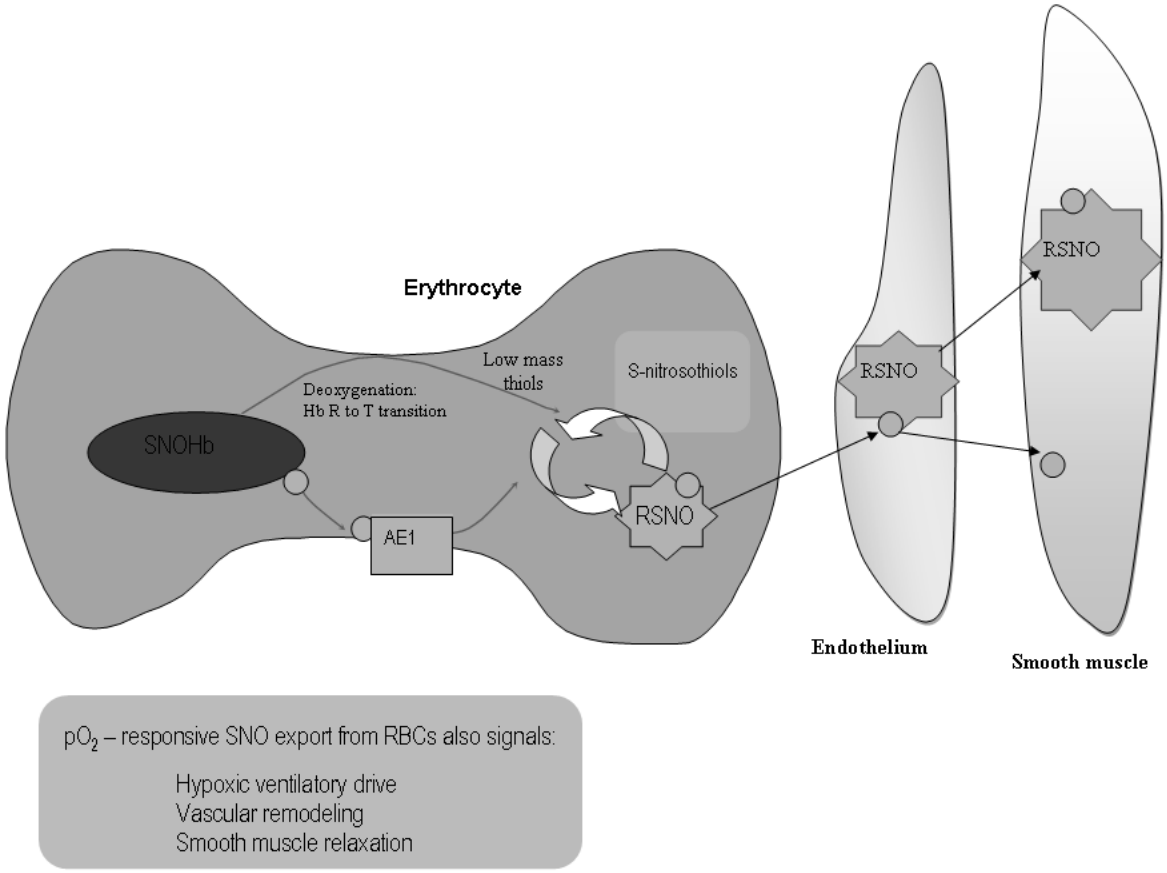

Figure. Transnitrosation signals hemoglobin (Hb) desaturation

Erythrocytes are endogenously pre-loaded with nitrosonium in the form of S-nitrosohemoglobin.[1-6,11,12,17] Oxyhemoglobin desaturation exposes the S-nitrosothiol bond of S-nitrosohemoglobin to transnitrosation reactions with endogenous and exogenous low mass thiols (RSH) and erythrocytic thiols (like anion exchange protein 1 [AE1])[14] during R to T conformational change.[1-6,11] Low mass S-nitrosothiols can enter endothelial cells.[5] where they S-nitrosylate specific protein targets, including gene regulatory proteins involved in vascular remodeling and in physiological regulation.[5,6] Also, through subsequent S-nitrosylation reactions and/or homolytic cleavage to NO, they can cause cGMP-dependent smooth muscle relaxation.[2]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript