S-Nitrosation of Hemoglobin and Hypoxic Pulmonary Vasoconstriction

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Hemoglobin (Hb) augments hypoxic pulmonary vasoconstriction (HPV) by scavenging nitric oxide (NO). However, S-nitrosation of Hb at the B-cysteine 93 residue may confer it with NO donor and vasodilatory properties. Furthermore, cross-linking of Hb may limit its vasoconstrictive potential by preventing abluminal movement. We studied the effects of Hb modification by S-nitrosation (SNO-Hb) and cross-linking at the B-cys 93 residue (Bis-Mal-PEG-Hb) in isolated, perfused rat lungs. HPV was defined by the change in pulmonary artery pressure (PAP) after ventilation with anoxic gas for 5 minutes, and PAP and HPV were compared before and after addition of human oxyHb, SNO-Hb, or Bis-Mal-Peg Hb (100 ΦM) to buffer perfusate. Exhaled NO (eNO) was measured by chemiluminescence. In a subgroup of experiments, the allosteric modulator inosine hexaphosphate (IHP) and glutathione (GSH) were added to perfusate containing SNO-Hb to promote release of NO from Hb, and to perfusate containing oxyHb to serve as a control.

Findings: All Hb preparations resulted in comparable increases in normoxic PAP and HPV (*$p<0.05$, buffer vs. Hb).

	PAP (Torr)		HPV (Torr)		eNO (ppb)	
Group	buffer	Hb	buffer	Hb	buffer	Hb
oxyHb	8.8∀0.5	10.0∀0.3*	1.6∀0.7	7.5∀1.9*	3∀1.0	3∀0.5
SNO-Hb	8.5∀0.8	9.6∀0.9*	2.2∀0.9	7.6∀2.7*	1.4∀0.6	1.0∀0.6
B-M-P-Hb	8.4∀0.6	11.4∀1.0*	2.8∀0.5	6.5∀1.0*	1.6∀0.5	1.4∀0.5

eNO was low during buffer perfusion, and did not change significantly with addition of any Hb preparation to the perfusate. HPV after addition of IHP to oxyHb was 3.3∀0.8, after addition of IHP to SNO-Hb 3.0∀1.1, and after addition of IHP plus GSH to SNO-Hb 3.2∀1.0 Torr.

Summary: Intramolecular cross-linking of Hb at the B-cysteine 93 residue does not ameliorate the vasoconstrictive properties of Hb in the lung. In addition, SNO-Hb, a putative systemic vasodilator, is a pulmonary vasoconstrictor and augments HPV. Augmentation of HPV by SNO-Hb occurred in both the absence and presence of GSH, suggesting that trans-nitrosation is not an important mechanism for NO delivery from Hb to the vascular smooth muscle in this model. An additional observation is that NO is exhaled in low concentration from rat lungs, and is not affected significantly by manipulation of perfusate, or circulatory, NO.

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