 Editorial

Endocrine Nitric Oxide Bioactivity and Hypoxic Vasodilation by Inhaled Nitric Oxide
Jonathan S. Stamler, James D. Reynolds, Douglas T. Hess

Inhaled nitric oxide (iNO) is approved by the Food and Drug Administration for the treatment of persistent pulmonary hypertension of the newborn, largely as a result of the pioneering research efforts of Warren Zapol. Inhaled nitric oxide (NO) dilates pulmonary resistance vessels to improve ventilation-perfusion matching before being inactivated by reactions with hemoglobin (Hb) in blood exiting the lung. Inhaled NO therefore is a selective pulmonary vasodilator.1 Notwithstanding the validity of this model, investigators including Claes Frostell2 and David Wessel3 noted early on that high therapeutic doses of NO led to subtle decreases in blood pressure. It had recently been discovered that albumin could be S-nitrosylated by NO,4 endowing it with long-lived vasodilatory activity. So, when presented with the quandary of systemic effects, it was quickly demonstrated that iNO could S-nitrosylate plasma proteins,5 potentially providing systemic vasodilatory activity6 and thus acting in an endocrine-like manner. S-nitrosoalbumin since has been implicated in protection by iNO against reperfusion injury in systemic vessels,7,8 but also may form within airways of iNO-treated patients,5,9 potentially contributing to “pulmonary selectivity.” In subsequent studies, the family of circulating S-nitrosylated proteins (SNO-proteins) was expanded to include S-nitrosohemoglobin (SNO-Hb),10 which exhibits the remarkable ability to mediate hypoxic vasodilation,11 the selective vasodilation in proportion to degree of hypoxemia. A number of recent studies have provided evidence that iNO may utilize SNO-based pathways12 to confer protection against ischemic insults13–18, none more remarkable than the report by Terpolilli et al19 in this issue of Circulation Research, which illustrates beautifully in cerebral vessels the delivery of NO bioactivity conveyed through SNOs is coupled to O2 delivery and thus is regulated by tissue PO2.1 Thus, Terpolilli et al19 not only demonstrate that iNO increases the levels of a naturally occurring vasodilator that functions to transduce O2 concentrations, but also actually record directly the in situ delivery of NO-based activity that subserves hypoxic vasodilation.

The finding that inhalation of NO leads to increases in SNO-Hb is consistent with the view that Hb functions to process endogenous nitrogen oxides into potent vasodilator nitrosothiols (SNOs),11,21 the only NO-based species that retain vasodilatory activity in the blood stream. The fact that NO synthase-derived NO10,11 (as well its metabolic byproduct nitrite22,23) can contribute to red blood cell-derived NO bioactivity in the form of SNO-Hb supports an expanded model of the respiratory cycle that is based on the coordinated transport of three gases, NO, O2, and CO2.21 In this cycle, the delivery of NO bioactivity conveyed through SNOs is coupled to O2 delivery and thus is regulated by tissue PO2. It has been shown that this process is governed by Hb allostery,14–24 that is, by changes in the quaternary conformation of Hb associated with changes in O2, CO2, and H+ concentrations. Hypoxia, hypercarbia, and acidosis promote the deoxygenated conformation (T-structure) in Hb that coordinately liberates SNO and O2, thereby matching blood flow with metabolic demand.24–25

In the study by Terpolilli et al,19 the increase in SNO-Hb induced by 50 ppm NO is modest, reflecting both caveats with the triiodide-based assay for SNO used,26 which greatly underestimates true levels, as well as the difficulty of generating SNOs using NO itself. Inhaled NO can generate SNO-Hb through multiple reaction channels,11,21,27,28 but all are relatively inefficient. In the first of these reactions, NO that reacts with hemes of Hb can transfer to the adjacent Cys93 residue. However, heme-to-thiol transfer of NO is constrained by a number of factors (Hb conformation, disposition of NO within the tetramer, redox requirements) and thus is effectively capped at low levels.11 SNO-Hb also can be formed from nitrite, but this too involves heme-to-thiol exchange reactions and thus cannot be scaled pharmacologically. A third pathway entails reactions of NO and O2 (and it is

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From the Institute for Transformative Molecular Medicine (J.S.S., J.D.R., D.T.H.), Department of Medicine (J.S.S., D.T.H.), and Department of Anesthesiology (J.D.R.), Case Western Reserve University, Cleveland, Ohio; University Hospitals Case Medical Center (J.S.S.), Cleveland, Ohio.

Correspondence to Jonathan S. Stamler, MD, Institute for Transformative Molecular Medicine, Wolstein Research Building 5522, 2103 Cornell Road, Cleveland, OH 44106. E-mail jonathan.stamler@case.edu

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Notable findings in this study include: (1) iNO increased circulating levels of SNO-Hb and the SNO-Hb-generating substrate, nitrite; (2) iNO selectively dilated pial venules under normoxic conditions and dilated both venules and arterioles under conditions of hyperperfusion and ischemic insult; and (3) neuroprotection by iNO was correlated with the delivery of NO to ischemic cerebral vessels and resultant vasodilation. It is known that SNO-Hb can actuate graded vasodilation in the brain across a wide range of oxygen tensions.20 Thus, Terpolilli et al19 not only demonstrate that iNO increases the levels of a naturally occurring vasodilator that functions to transduce O2 concentrations, but also actually record directly the in situ delivery of NO-based activity that subserves hypoxic vasodilation.

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notable that Terpolilli et al19 coadministered iNO and O2, highlighting the intermediacy of nitrogen oxides that can generate SNOs. In one scheme, S-nitrosoglutathione produced in airways from N2O3 is envisioned to generate SNO-Hb through a simple transnitrosylative transfer of the NO group. Although S-nitrosoglutathione has been measured in airways of patients receiving iNO,5 extraneous reactions of NO group. Although S-nitrosoglutathione has been measured in airways of patients receiving iNO,5 extraneous reactions of

The delivery of vasoregulatory SNO-based bioactivity to peripheral sites, including the brain, may be described as a set of equilibria whereby SNO generation at a site of origin is coupled to the release of bioactivity in tissues. Release of SNO-based bioactivity may be regulated by a number of physiological stimuli, and the response to hypoxic stimulation is conveyed primarily by SNO-Hb.

Low yields of these reactions aside, there is still something rather remarkable about the fact that increasing SNO levels in the lungs results in increased SNO levels in the brain19 (this property is not unique to NO bioactivity generated in the lung, because NO bioactivity generated in skeletal muscle is also delivered to the brain20). One interpretation of these data is that SNOs in the lung may be viewed as part of an integrated system in which the relationship between SNOs in the lung, blood, and peripheral vasculature, including that of the brain, represents a set of coupled equilibria (Figure). Under this model, delivery of NO bioactivity via sequential transnitrosylation reactions represents a shift across these equilibria that would allow for the rapid delivery required for physiological signaling on short time scales—in effect, a “bucket brigade” whereby an incoming NO group at one end displaces an NO group at the other end, by analogy to the flow of electrons in conducting wires or the movement of protons in water. Thus, there would be no requirement that the NO molecule picked up in the lung is the same as that delivered in the tissue.

Misconceptions surrounding the possible role and function of nitrite merit comment. Nitrite is relatively inert, being orders of magnitude less potent as a vasodilator than SNOs. Nitrite at concentrations observed in the circulation under normal physiological conditions—and in the amounts observed after administration of iNO in this study—does not appear to exert biological activity in its own right.25 Claims of nitrite-induced vasodilation under physiological (as opposed to pharmacological) conditions are not well-justified, and although the case for nitrite in NO-based signaling under ischemic conditions may be stronger on both empirical and mechanistic grounds, ischemic enhancement of the vasoreactive potency of nitrite is inevitably commensurate with production of SNOs. Indeed, in cells and tissues, nitrite-forming reactions will almost always produce SNOs and the generation of NO bioactivity from nitrite will almost inevitably involve SNOs.15,23,30 Thus, the evidence for an alternative nitrite-based but SNO-independent mechanism of hypoxic vasoregulation in ischemia is, at best, circumstantial and the notion that nitrite may operate independently of SNOs is difficult to sustain.25 In addition, nitrite-based vasodilatory activity often is claimed to involve a nitrite reductase activity of Hb or xanthine oxidase, which reduces nitrite to NO.19 However, the available data would seem to rule out a role for a nitrite reductase activity of Hb in vasodilation,25,31 and xanthine oxidase evidently exerts a vasoconstrictive rather than vasodilatory influence in humans.23,33 With the exception of the SNO synthesis function of Hb,21 an enzymatic activity that may promote the vasodilatory activity of iNO remains to be identified.

Measurements of NO bioactivity in the blood of Tibetans show that levels of SNO-Hb are markedly increased compared with those of sea-level dwellers, commensurate with increases in peripheral blood flow.34 Additional potential benefits of SNO-Hb include roles in ventilation–perfusion matching in the lung and the centrally mediated hypoxic respiratory drive.21 Notably, SNO-Hb levels increase in normal subjects acclimatizing to high altitude, and levels of both Hb and SNO-Hb predict distance walked at 6 minutes.34 Human blood gas measurements show that O2 content (O2 saturation) of Hb is intimately linked to its SNO content,36,37 and recent studies highlight the centrality of S-nitrosylation–based signaling in transducing ambient O2 signals in both anemic and hypoxic conditions.38,39 Taken together, these observations emphasize that O2 delivery within the respiratory cycle is a function of both O2 content of blood (Hb O2 saturation) and blood flow (tissue perfusion, regulated by SNO-Hb). Current efforts in medicine to improve O2 delivery are focused on O2 content of blood without consideration of SNO bioactivity. In contrast, the findings of Terpolilli et al19 suggest therapeutic opportunities for iNO operating through SNO-based mechanisms, particularly in the setting of cerebral ischemia. As important, their findings point to the importance of developing new strategies that more effectively manipulate NO-based vasoactivity through SNO-based pathways.

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