How Does Inhaled Nitric Oxide Reach Peripheral Tissues?

Paul Kubes

Summary. Conventional wisdom would dictate that nitric oxide is a local autocoid with spatially limited effects. Over the last few years, we, and others have challenged this view and have used inhaled nitric oxide to demonstrate that despite its administration in lung, it can impact the peripheral vasculature. This chapter summarizes some of the evidence to support the contention that nitric oxide can impact peripheral vasculatures presumably via a stabilizing moiety in the circulation. One possibility is the formation of *S*-nitrosothiols, which extend the half-life of nitric oxide many-fold. In this chapter I provide evidence that *S*-nitrosothiols exist in the vasculature, particularly during nitric oxide inhalation. Finally, I highlight the limited evidence for the role that these potent vasodilating molecules may play as physiologically and therapeutically important regulators of the vascular system.

Key words. Nitrosothiol, *S*-Nitrosoalbumin, Ischemia/reperfusion, Microcirculation

Introduction

For many years the diatomic free radical nitric oxide was considered exclusively as an atmospheric pollutant produced during the combustion of fossil fuels [1,2]. It is now well appreciated that nitric oxide is produced by a large variety of organisms including all mammals [3–5]. Nitric oxide has many essential important functions including blood pressure regulation, host

Immunology Research Group, Department of Physiology and Biophysics, University of Calgary, Health Sciences Center, 3330 Hospital Drive NW, Calgary, AB, T2N 4N1, Canada

defense, and neurotransmission [4,6]. However, conventional wisdom dictates that these effects are mediated locally, by NO-producing cells. In fact, NO is rapidly inactivated by oxyhemoglobin (HbO_2, i.e., $Hb[Fe_{II}]O_2$) to form methemoglobin (MetHb, i.e., $Hb[Fe_{III}]$) and nitrate (NO_3^-) within the bloodstream, thereby restricting its actions to the site of production. Herein, I describe the possibility that in fact nitric oxide may be stabilized in the circulation and may have much further-reaching effects than was previously proposed.

RSNOs, Vasodilators Formed in Blood

Loscalzo, Stamler, and other colleagues challenged the view that nitric oxide was restricted to local effects when they proposed that nitric oxide can potentially bind carrier molecules to form nitric oxide adducts called *S*-nitrosothiols (RSNOs). These molecules were shown to function as nitric oxide-carrying systems, prolonging the half-life and spatial impact of nitric oxide [7,8]. First, plasma RSNOs have been detected in vivo by many investigators [7–13]. Examples of RSNOs include low molecular weight *S*-nitrosocysteine (CysNO), *S*-nitrosoglutathione (GSNO), and high molecular weight *S*-nitroso-albumin (SNO-Alb). Among these RSNOs, SNO-Alb tends to be more stable than low molecular weight molecules [7] and is the principal molecule formed [7]. Injection of any of these RSNOs into animals results in prolonged vasodilation [7,8,14,15]. Therefore, RSNOs can be formed in vivo and when synthesized ex vivo and injected into animals, they have vasodilating properties. The question that remained was whether RSNO can be produced in sufficient quantities in vivo to function as circulating vasodilators.

Inhaled Nitric Oxide Affects Peripheral Vascular Beds

Nitric oxide is administered directly by inhalation for the treatment of pulmonary hypertension in newborn infants [16,17] and acute respiratory distress syndrome in adults [4,16]. Although this form of nitric oxide delivery is used for regional impact exclusively on the pulmonary vasculature, we made use of this system to ask whether the nitric oxide administered in the lung could exert its biology beyond the lung. This would indeed support the production of NO carriers in vivo. However, our initial attempts failed miserably. Inhalation of 80 ppm nitric oxide did not in any way affect blood pressure or, for example, intestinal blood flow. This was consistent with similar reports by others that inhalation of nitric oxide does not affect basal physiology. However, when nitric oxide was systemically inhibited and systemic blood pressure was increased, inhaled nitric oxide reduced but did not prevent the

rise in blood pressure. However, the problem with this experiment was that the systemic changes in blood pressure could simply reflect changes within the pulmonary microvasculature. Therefore, Fox-Robichaud and colleagues next decided to inhibit nitric oxide production locally within a small distal microvasculature [18]. This was accomplished by superfusing the mesenteric microvasculature and using intravital microscopy to visualize changes within the microvessels of the mesentery. When nitric oxide was inhibited, profound increase in vasoconstriction and leukocyte adhesion was noted in the mesenteric microvessels. When animals were made to breath inhaled nitric oxide, the vasoconstriction was no longer detectable and leukocyte adhesion was greatly reduced. Clearly, delivery of nitric oxide at the lung, somehow affected the mesenteric microvessels.

One potential criticism of this work was that at least the leukocyte effects could potentially have occurred within the lung making the leukocytes no longer adhere in the periphery. To address whether the leukocytes were being affected within the lung, the animals were made to breathe nitric oxide, and their blood was immediately taken and perfused through a flow chamber containing adhesion molecules (surrogate blood vessel). The data from this series of experiments clearly revealed that leukocytes adhered as effectively when taken from animals breathing room air or nitric oxide [18]. Clearly the nitric oxide was not affecting the leukocytes, but rather was reaching the distal microvasculature and modulating the microvessels. Indeed, this conclusion was also consistent with the inhibition of vasoconstriction in the peripheral blood vessels with inhaled nitric oxide wherein leukocytes were presumably not involved. Moreover, the vasoconstriction data were reproduced in humans. Responses to inhibition of local NO synthesis was reversed following administration of inhaled NO [18,19].

Inhaled Nitric Oxide Affects Pathophysiology in Peripheral Organs

Although the reversal of nitric oxide inhibition by inhaled nitric oxide was important support that nitric oxide could reach peripheral vasculatures, from a therapeutic standpoint, it remained unclear whether this approach could improve pathology. Fox-Robichaud and colleagues tested this hypothesis and demonstrated that inhaled NO was also beneficial in pathological conditions wherein NO has been documented to be reduced. Inhaled NO reduced leukocyte recruitment and prevented reductions in blood flow caused by ischemia/reperfusion [18,20]. Interestingly, when the mesentery was exposed to lipopolysaccharide which induced profound leukocyte recruitment,

inhaled nitric oxide was not able to reduce this adhesion [18]. The difference between the two models was that in ischemia/reperfusion nitric oxide is low or completely inhibited whereas in the endotoxemia model increased NO production is a key feature. Clearly, inhaled nitric oxide will not be beneficial in all vascular diseases.

The next important question was whether there was an increase in RSNOs during NO inhalation. Although both Cannon and colleagues and Kubes and colleagues observed an increase in NO-carrying molecules, the specific molecules detected were quite different. Cannon and colleagues [19] demonstrated that HbNO in blood rose significantly during NO breathing, they were not able to detect a change in SNO-Alb in plasma with NO inhalation. This is in contrast to our own data wherein we did observe an increase in SNO-Alb (Ng and Kubes (2004) Circ Res 94(4):559–565). A very likely explanation for this difference, which in fact was raised by Cannon and colleagues, is that SNO-Alb decomposes rapidly due to the interaction of this species with low molecular weight thiol groups [19]. Addition of sulfanilamide, diethylenetriaminepentaacetic acid (DTPA), and *N*-ethylmaleimide during sampling can reduce SNO-Alb degradation and transnitrosation reactions.

Other investigators have delivered nitric oxide directly into the bloodstream (not through inhalation) and also observed RSNO formation. Marley and colleagues [21] studied the formation of RSNOs from low fluxes of NO in plasma. They showed that significant amounts of RSNOs and more specifically, SNO-Alb were formed. Rassaf and colleagues [22] also demonstrated the formation of plasma RSNOs in vivo following infusion of NO into the bloodstream. Again more than 90% of the high molecular weight RSNOs were SNO-Alb. Rassaf et al. [22] observed both a rapid vasodilation consistent with administration of exogenous NO and a delayed vasodilation temporally similar to effects observed with the administration of RSNOs.

Another important question was whether inhaled NO was restricted to the microvasculature or whether it was also able to impact on sites outside the vasculature. Inhibition of nitric oxide in the intestine caused an increase in epithelial or mucosal permeability [20]. However, inhaled nitric oxide was unable to reduce the increased mucosal permeability whereas NO donors did have biological activity at these extravascular sites. These data suggest that the NO delivery system was restricted to the vasculature. When lymphatics were cannulated and the RSNOs measured, there was absolutely no notable increase in these molecules. Clearly, these observations suggest that RSNOs can form in the vasculature but they are unable to reach the extravascular space. One possibility is that the nitric oxide is removed from the RSNO by endothelium or that the main RSNO is too large to reach the extravascular space. One example of this would be SNO-hemoglobin inside red blood cells, which do not enter the extravascular space.

How Might RSNOs Be Formed In Vivo?

As a free radical, NO is highly reactive and short-lived with a half-life of only 0.05–1 s in blood [7,23,24]. Although NO is thought not to react directly with thiols, a number of indirect reactions for RSNO formation have been proposed. In the aqueous phase of plasma, NO may react with O_2 to form higher oxides of nitrogen (N_2O_3), which subsequently leads to the formation of nitrite (NO_2^-). On the other hand, the intermediate, N_2O_3 (a carrier of NO^+), formed from the autoxidation of NO, may undergo *S*-nitrosylation with molecules containing thiol groups to form RSNOs [8,25,26]. Although a number of these reactions have been demonstrated in vitro, the exact mechanisms by which RSNOs are formed in vivo remain uncertain due to the complexity of blood leading to numerous other competitive reactions in biological systems [21,25,27].

Nitric oxide interacts with superoxide (O_2^-) to form peroxynitrite ($ONOO^-$) in plasma. $ONOO^-$ decomposes rapidly once protonated to generate either nitrate (NO_3^-) [23,24,28] or strong oxidants such as hydroxyl and nitrogen dioxide radicals. Reactions between $ONOO^-$ and thiols have been reported leading to the formation of RSNOs [29–33]. The observation that administration of exogenous $ONOO^-$ into animals induced responses reminiscent of RSNOs including vasodilation [29,33] and inhibition of leukocyte–endothelial cell interactions [34] potentially supports the view that $ONOO^-$ rapidly reacts with thiols to form RSNOs.

Finally, metabolic pathways of NO in erythrocytes other than to form nitrate via oxyhemoglobin may function as important NO delivery systems. For example, a small proportion of NO may bind to deoxyhemoglobin (Hb, i.e., $Hb[Fe_{II}]$) to form nitrosylhemoglobin (HbNO, i.e., $Hb[Fe_{II}]NO$), or with the 93-cysteine residue of the β-subunit to form *S*-nitrosohemoglobin (SNO-Hb) [24,35–37]. The fraction of each is dependent on the ratio of oxygenated and deoxygenated Hb within the erythrocytes [23,24]. These molecules have been detected in vivo and can induce vasodilation when formed exogenously in red blood cells and reinjected into animals. The question that remains is whether sufficient amounts of these hemoglobin molecules are produced in vivo to affect peripheral vasculatures.

Concluding Remarks

There is a growing body of evidence that nitric oxide is not a local autocoid that influences only its immediate environment. It is now becoming apparent that nitric oxide may combine with thiols and be transported around the circulatory system, perhaps delivering nitric oxide where it is needed most.

Inhaled nitric oxide is a simple and effective tool to begin to demonstrate that nitric oxide can indeed bind molecules within the blood to impact extrapulmonary vasculatures. Since inhaled nitric oxide is a reasonably safe method for nitric oxide delivery, it could potentially be important as a therapeutic modality in various cardiovascular diseases.

References

1. Burgner D, Rockett K, Kwiatkowski D (1999) Nitric oxide and infectious diseases. Arch Dis Child 81:185–188
2. Keh D, Gerlach H, Falke K (2000) Inhalation therapy with nitric oxide gas. In: Mayer B (ed) Nitric oxide. Springer, Berlin Heidelberg New York, pp 399–441
3. Ignarro LJ, Buga GM, Wood KS, et al (1987) Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci USA 84:9265–9269
4. Moncada S, Higgs A (1993) The L-arginine-nitric oxide pathway. N Engl J Med 329: 2002–2012
5. Palmer RMJ, Ferrige AG, Moncada S (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327:524–526
6. Moncada S, Palmer RMJ, Higgs EA (1991) Nitric oxide: physiological, pathophysiology, and pharmacology. Pharmacol Rev 43:109–142
7. Stamler JS, Jaraki O, Osborne J, et al (1992) Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin. Proc Natl Acad Sci USA 89:7674–7677
8. Stamler JS, Simon DI, Osborne JA, et al (1992) S-nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds. Proc Natl Acad Sci USA 89:444–448
9. Goldman RK, Vlessis AA, Trunkey D (1998) Nitrosothiol quantification in human plasma. Anal Biochem 259:98–103
10. MacMicking J, Xie QW, Nathan C (1997) Nitric oxide and macrophage function. Annu Rev Immunol 15:323–350
11. Marzinzig M, Nussler AK, Stadler J, et al (1997) Improved methods to measure end products of nitric oxide in biological fluids: nitrite, nitrate, and S-nitrosothiols. Nitric Oxide Biol Chem 1:177–189
12. Minamiyama Y, Takemura S, Inoue M (1997) Effect of thiol status on nitric oxide metabolism in the circulation. Arch Biochem Biophys 341:186–192
13. Scharfstein JS, Keaney JF, Slivka A, et al (1994) In vivo transfer of nitric oxide between a plasma protein-bound reservoir and low molecular weight thiols. J Clin Invest 94: 1432–1439
14. Myers PR, Minor RL Jr, Guerra R Jr, et al (1990) Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble S-nitrosocysteine than nitric oxide. Nature 345:161–163
15. Rubanyi GM, Johns A, Wilcox D, et al (1991) Evidence that a S-nitrosothiol, but not nitric oxide, may be identical with endothelium-derived relaxing factor. J Cardiovasc Pharmacol 17:S41–S45

16. Kinsella JP, Neish SR, Shaffer E, et al (1992) Low-dose inhalational nitric oxide in persistent pulmonary hypertension of the newborn. Lancet 340:819–820
17. Roberts JD, Polaner DM, Lang P, et al (1992) Inhaled nitric oxide in persistent pulmonary hypertension of the newborn. Lancet 340:818–819
18. Fox-Robichaud A, Payne D, Hasan SU, et al (1998) Inhaled NO as a viable antiadhesive therapy for ischemia/reperfusion injury of distal microvascular beds. J Clin Invest 101:2497–2505
19. Cannon RO III, Schechter AN, Panza JA, et al (2001) Effects of inhaled nitric oxide on regional blood flow are consistent with intravascular nitric oxide delivery. J Clin Invest 108:279–287
20. Kubes P, Payne D, Grisham MB, et al (1999) Inhaled NO impacts vascular but not extravascular compartments in postischemic peripheral organs. Am J Physiol (Heart Circ Physiol) 277:H676–H682
21. Marley R, Patel RP, Orie N, et al (2001) Formation of nanomolar concentrations of S-nitroso-albumin in human plasma by nitric oxide. Free Radic Biol Med 31:688–696
22. Rassaf T, Preik M, Kleinbongard P, et al (2002) Evidence for in vivo transport of bioactive nitric oxide in human plasma. J Clin Invest 109:1241–1248
23. Kelm M (1999) Nitric oxide metabolism and breakdown. Biochim Biophys Acta 1411: 273–289
24. Kelm M, Yoshida K (1996) Metabolic fate of nitric oxide and related N-oxides. In: Feelisch M, Stamler JS (eds) Methods in nitric oxide research. Wiley, West Sussex, pp 47–58
25. Kharitonov VG, Sundquist AR, Sharma VS (1995) Kinetics of nitrosation of thiols by nitric oxide in the presence of oxygen. J Biol Chem 270:28158–28164
26. Stamler JS, Singel D, Loscalzo J (1992) Biochemistry of nitric oxide and its redox-activated forms. Science 258:1898–1902
27. Nedospasov A, Rafikov R, Beda N, et al (2000) An autocatalytic mechanism of protein nitrosylation. Proc Natl Acad Sci USA 97:13543–13548
28. Beckman JS, Beckman TW, Chen J, et al (1990) Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 87:1620–1624
29. Liu S, Beckman JS, Ku DD (1994) Peroxynitrite, a product of superoxide and nitric oxide, produces coronary vasorelaxation in dogs. J Pharmacol Exp Ther 268:1114–1121
30. Moro MA, Darley-Usmar V, Goodwin DA, et al (1994) Paradoxical fate and biological action of peroxynitrite on human platelets. Proc Natl Acad Sci USA 91:6702–6706
31. Nossuli T, Hayward R, Scalia R, et al (1997) Peroxynitrite reduces myocardial infarct size and preserves coronary endothelium after ischemia and reperfusion in cats. Circulation 96:2317–2324
32. van der Vliet A, Chr't Hoen PA, Wong PSY, et al (1998) Formation of S-nitrosothiols via direct nucleophilic nitrosation of thiols by peroxynitrite with elimination of hydrogen peroxide. J Biol Chem 273:30255–30262
33. Wu M, Pritchard KA Jr, Kaminski PM, et al (1994) Involvement of nitric oxide and nitrosothiols in relaxation of pulmonary arteries to peroxynitrite. Am J Physiol (Heart Circ Physiol) 266:H2108–H2113
34. Lefer DJ, Scalia R, Campbell B, et al (1997) Peroxynitrite inhibits leukocyte-endothelial cell interactions and protects against ischemia-reperfusion injury in rats. J Clin Invest 99:684–691

35. Gow AJ, Stamler JS (1998) Reactions between nitric oxide and hemoglobin under physiological conditions. Nature 391:169–173
36. Jia L, Bonaventura C, Bonaventura J, et al (1996) S-nitrosohemoglobin: a dynamic activity of blood involved in vascular control. Nature 380:221–226
37. Stamler JS, Jia L, Eu JP, et al (1997) Blood flow regulation by S-nitrosohemoglobin in the physiological oxygen gradient. Science 276:2034–2037