

6. Experimental studies on nitric oxide

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This chapter covers pharmacological and toxicologic studies on effects of exposure to nitric oxide (NO), by way of inhalation in humans and animals. Focus has been kept on exposure to low concentrations or on mechanistically interesting studies. A brief review of the formation of NO in the body, endogenous NO, is also given, since knowledge of endogenous NO offers new insights to possible mechanisms for NO action. A third form of tissue exposure to NO comes from formation in the body from organic nitrites and nitrates, for example, amyl nitrite and nitroglycerin (1, 2).

Endogenous formation

In cells of many of the organ systems, such as nerve tissue, blood vessels and the immune system, endogenous NO synthesis occurs by NO formation from physiological substrate (the amino acid L-arginine). Thus NO has been found to be produced by at least three different oxygen-utilizing NO synthases, for purposes such as signaling in the nervous system, mediating vasodilation in both systemic and pulmonary circulation, and mediating cytotoxicity and host defense reactions in the immune system (3-8). The impact of these findings for an understanding of the toxicologic effects of NO is still difficult to assess, but clearly a new basis for NO pharmacology and toxicology might be at hand. The actions of endogenous NO can be divided into two main groups.

The first group involves low concentrations of NO (nano- to picomolar) formed by constitutive enzymes in nerve and endothelial cells. This NO has local discrete actions exerted via activation of an enzyme, guanylate cyclase, in the target cell (9).

The second group involves high concentrations of NO (micro- to nanomolar) formed by enzymes that can increase in amount through the induction of enzyme upon exposure to bacterial toxins or to growth-regulating factors (cytokines). The induction of such NO synthesis can be counteracted by corticosteroids. The inducible NO formation occurs especially in macrophages and neutrophil leukocytes and is important for the killing of bacteria and parasites, and possibly also for cytostasis in antitumor reactions (7, 10).

For the effects of inhaled NO it is important to consider that endogenous NO regulates pulmonary

vascular resistance; it is found in small amounts in exhaled air and has been suggested to be necessary for normal oxygenation of the blood (11, 12). In agreement, the inhalation of NO by healthy individuals evokes pulmonary vasodilation (13).

Effects of exposure

Toxicologic data on NO effects on humans or animals are not abundant, and generally NO at concentrations found in ambient air seems to have little adverse effects apart from methemoglobin formation, unless NO is contaminated with or transformed to nitrogen dioxide (NO₂). Indeed, recent clinical studies have generally shown positive effects of NO on lung function, and suggested guidelines for such studies, in order to minimize NO₂ exposure, have been published (14). However, a few experimental studies indicate temporary adverse effects from the inhalation of moderate concentrations of NO.

Uptake

NO is not very well absorbed by ventilated isolated perfused lung (15), probably due to its modest solubility in aqueous media. In contrast, it is absorbed to a large extent in normally breathing subjects (85-92% at 400 to 6100 µg·m⁻³) (16, 17), a phenomenon probably reflecting the rapid uptake over the alveolar membrane and the subsequent reaction of NO with hemoglobin (17). From auto exhaust mixtures, 73% of the NO was adsorbed in the upper airway mucosal membranes of dogs (18).

Nitric oxide and lethality

Acute exposure to high concentrations of NO has been found to be lethal, and contribution to the lethality might have been exerted by NO₂ being generated during the exposure. Thus dogs exposed to 24 540 µg·m⁻³ (20 000 ppb) for 7 to 50 min died within 15 min, and one animal challenged with 5000 ppb also died (19), the cause of death being pulmonary edema. A 50% mortality was observed for mice in an early study on mice exposed to NO at 392 000 µg·m⁻³ (320 ppm) for 8 h (20). No increase in death rate was found for mice exposed to 12 270 µg·m⁻³ (10 ppm) for 6.5 months (21) or to 2940 µg·m⁻³ (2.4 ppm) for their entire life span (23 to 29 months) (22).

Effects on pulmonary function

No change in respiratory function was found in guinea pigs exposed to NO at 19 600 $\mu\text{g}\cdot\text{m}^{-3}$ (16 ppm) or 61 300 $\mu\text{g}\cdot\text{m}^{-3}$ (50 ppm) for 4 h (23). Increased airway responsiveness to acetylcholine was observed in guinea pigs exposed 2 x 30 min per week for seven weeks to 6130 $\mu\text{g}\cdot\text{m}^{-3}$ (5 ppm). In sheep, significant reversal of vasoconstriction to an infused thromboxane analog was seen in acute NO exposure of 5 ppm (24). Also in sheep, hypoxic vasoconstriction was significantly diminished by acute exposure to 5 ppm of NO, and it was nearly abolished at 40 ppm in inhaled air (25). In human volunteers, moderate bronchoconstriction was seen at 20 ppm and above (26). Kagawa (27) exposed healthy humans to 1 ppm of NO for 2 h, with intermittent periods of moderate exercise. In half of the subjects, a small but significant decrease in airway conductance was seen, and, as a group, a significant decrease in maximal expiratory flow occurred when a mixture of helium and oxygen was breathed. A contribution from NO₂ might be one explanation for these effects, or bronchial congestion due to vasodilation by NO in the mucosa might have occurred (28).

In healthy workers, pulmonary vasodilation (measured as the reversal of hypoxic vasoconstriction), without any bronchoconstrictor effect, occurred at an NO level of 10 ppm and was marked at 40 ppm (13). These data might suggest that only exposure to high concentrations of NO causes vascular effects in the lung, but it is possible that this phenomenon is due to the presence of endogenous NO in normal healthy individuals and that the situation might be different in cases in which endogenous NO formation is diminished. Accordingly, administering an inhibitor of endogenous NO formation evokes vasoconstriction in the pulmonary circulation of rabbits, and this vasoconstriction is significantly attenuated by an NO level of 0.15 ppm and is fully reversed by 3-9 ppm in inhaled air (29). In untreated animals, vasodilator effects first occur at 9 ppm. It is not known which conditions in humans might exhibit depressed NO formation and thus enhanced sensitivity to exogenous NO. However, NO is clearly present in the exhaled air of humans (12), and it attains levels of 0.1-0.3 ppm in exhaled breath after breath holding (30). An important future issue will be whether vasodilator effects of inhaled NO should be regarded as substitution for endogenous NO and is thus beneficial or whether it might be regarded as an untoward action.

Relaxation of bronchial smooth muscle can be exerted *in vitro* by mechanisms dependent on an intact airway epithelium. An endogenous muscle-relaxing factor released by the epithelium has been suggested, but it is not clear whether it might be endogenous NO (31). Reversal of metacholine-induced bronchoconstriction by NO has been reported in guinea pigs already at 5 ppm (32), and in rabbits full reversal of

metacholine bronchoconstriction was at hand at 80 ppm (33). Preliminary data on humans (Höglman et al, to be published) show a moderate bronchodilator effect of 80 ppm (102 400 $\mu\text{g}/\text{m}^3$).

Effects on lung morphology

NO can cause morphological alterations in the lungs of experimental animals, but only at concentrations much higher than the NO₂ levels that elicit significant effects. Thus NO exposure of 2950 $\mu\text{g}\cdot\text{m}^{-3}$ (2.4 ppm) for 23-29 months had no effect on lung morphology among mice (34), and exposure of rats to 2460 $\mu\text{g}\cdot\text{m}^{-3}$ (2 ppm) continuously for six weeks elicited slight emphysema-like alterations in alveoli (35). Exposure to a combination of NO (2.05 mg m^{-3}) and NO₂ (0.27 mg m^{-3}) evoked a significant increase in interalveolar pores in mongrel dogs (36). Edema and thickening of the alveolocapillary membrane and vacuolization of endothelial cells were found in rabbits exposed to an NO concentration of 6150 $\mu\text{g}\cdot\text{m}^{-3}$ (5 ppm) continuously for 14 d (37), whereas in mice 12 300 $\mu\text{g}\cdot\text{m}^{-3}$ (10 ppm) 2 h daily, 5 d a week for 30 months (38), or continuously for 6.5 months (21), elicited changes in alveolar septa, congestion, enlarged peripheral air spaces and some changes in bronchiolar morphology. During combined exposure to NO (43 ppm) and NO₂ (3.6 ppm), Hugod (39) found no changes in alveolar cell morphology among rabbits, but swelling of endothelial cells occurred in both the exposed and the control group. These findings illustrate the difficulties inherent in this type of study.

Effects on host defense

Mice exposed to NO at 12 270 $\mu\text{g}\cdot\text{m}^{-3}$ (10 ppm) for 2 h a day, 5 d a week and up to 30 weeks (38) showed leukocytosis at five weeks and a decrease in red blood cell hemoglobin at 30 weeks. Altered responses to challenge with phytohemagglutinin in sheep red blood cells, as well as a decreased ability to reject virus-induced tumors, was seen at different stages of the exposure. In another study, male and female mice were continuously exposed to 2450 $\mu\text{g}\cdot\text{m}^{-3}$ (2 ppm) for 6 h to 4 weeks and tested for resistance to a bacterial aerosol (40). Only female animals showed a somewhat increased mortality and a decrease in survival time.

Metabolic effects

Mice exposed to NO concentrations of 12 300 to 25 800 $\mu\text{g}\cdot\text{m}^{-3}$ (10 to 21 ppm) for 3 h daily for 7 d showed no change in the levels of reduced glutathione in their lungs (41). *In vitro* data indicate that NO stimulates guanylate cyclase and therefore leads to smooth muscle relaxation and vasodilation and functional effects on the nervous system (3, 7, 9, 42).

These effects are probably responsible for vasodilation in the pulmonary circulation and an acute bronchodilator effect of inhaled NO, but it is unclear whether other effects might be exerted from ambient NO via this pathway. Due to the rapid inactivation of NO in hemoglobin, internal organs other than the lungs are unlikely to be affected directly by cyclic GMP (guanosine monophosphate)-mediated vasodilator influence from ambient concentrations of NO.

Methemoglobin formation, via the formation of nitrosylhemoglobin (22, 34, 43-46) and subsequent oxidation with oxygen, is well known (47, 48). During NO exposure of mice to 24 500 - 98 200 $\mu\text{g}\cdot\text{m}^{-3}$ (20-80 ppm), the levels of methemoglobin were found to increase exponentially with the NO concentration (34), and after the cessation of NO exposure methemoglobin decreased rapidly, with a half-time of a few minutes only. In humans the ability to reduce methemoglobin varies genetically and is lower in infants. Even so, when methemoglobin occurs due to NO, the levels can be decreased by lowering the concentration of NO during ongoing exposure (49). Of the NO reaction products with hemoglobin, methemoglobin attains higher levels than nitrosyl-hemoglobin (50). Exposure of mice to 2940 $\mu\text{g}\cdot\text{m}^{-3}$ (2.4 ppm) for 23-29 months resulted in nitrosylhemoglobin levels at 0.01%, while the maximal methemoglobin level was 0.3% (34). At 12 300 $\mu\text{g}\cdot\text{m}^{-3}$ (10 ppm) for 6.5 months, the nitrosyl-hemoglobin level was 0.13% and the level of methemoglobin was 0.2% (21). Rats exposed to 2450 $\mu\text{g}\cdot\text{m}^{-3}$ (2 ppm) continuously for six weeks showed no detectable methemoglobin (35).

Hematologic changes

Mice exposed to NO at 11 070 $\mu\text{g}\cdot\text{m}^{-3}$ (9 ppm) for 16 h had decreased iron transferrin (45), and exposure to 12 300 $\mu\text{g}\cdot\text{m}^{-3}$ (10 ppm) for 6.5 months increased the blood white cell count and the proportion of polymorphonuclear cells in a comparison with lymphocytes (21). It also affected red blood cell morphology, spleen weight, and bilirubin. A slight increase in hemolysis was seen in mice exposed to 2940 $\mu\text{g}\cdot\text{m}^{-3}$ (2.4 ppm) (22).

Nitric oxide application in lung disease

With the background of the aforementioned vasodilator effects of NO on pulmonary circulation, NO has recently been tried as a selective pulmonary vasodilator in patients with severe derangements of pulmonary ventilation or perfusion matching manifested either as hypoxia or increased pulmonary vascular resistance or both. Marked improvements occurred in these parameters, and, apart from moderate methemoglobinemia, no side effects have been reported during NO exposure of 2-20 ppm (49, 51-54). These results with humans are supported by findings of improved ventilation perfusion matching in

experimental animals exposed to NO (55).

Biochemical mechanisms for nitric oxide effects
Reaction with iron and effects on enzymes and nucleic acids. NO has an affinity to heme-bound iron which is 200-fold higher than that of carbon monoxide. This affinity leads to the formation of methemoglobin and the stimulation of guanylate cyclase that have already been discussed. Furthermore, NO reacts with thiol-associated iron in enzymes and eventually displaces the iron. This is a possible mechanism for the cytotoxic effects of NO (10, 56). In vitro, the NO donor sodium nitroprusside has been shown to mobilize iron from ferritin (57). NO might, possibly also via interference with iron, modulate arachidonic acid metabolism (58, 59).

NO inhibits aconitase, an enzyme in the Krebs cycle, and also complex 1 and 2 of the respiratory chain (10, 11, 60). Permanent modification of hemoglobin, possibly via deamination, has been found (61). NO can also deaminate DNA (deoxyribonucleic acid), and evoke DNA chain breaks and the inhibition of DNA polymerase and ribonucleotide reductase (62-65). In rat spleen cells NO might be antimitogenic and inhibit T-cell proliferation (66), and in vascular tissue NO donors inhibit DNA synthesis, cell proliferation, and mitogenesis (67, 68). ADP (adenosine diphosphate) ribosylation is stimulated by NO-generating agents (68).

Formation of peroxynitrite and other radicals. In biological systems in vitro, NO has been found to react with superoxide (O_2^-) to form peroxynitrite. Subsequently, the peroxynitrite can permanently modify proteins via the formation of nitrotyrosine (70). Further reactions might involve the formation of OH radicals, lipid peroxidation, and the oxidation of thiols (71-74). Methods have been developed for determining nitrotyrosine and its metabolites, and these agents can be demonstrated in the urine of humans, possibly also as a measure of NO_2 exposure (75, 76). The modification of tyrosine affects its ability to be phosphorylated, an important reaction for the regulation of protein function (77).

The potential of these mechanisms might be that they offer explanations for the cytotoxic effects of NO in host-defense reactions, but they may also explain the adverse effects of elevated endogenous levels of NO (eg, in conjunction with ischemic episodes), as well as effects from NO exposure.

Summary and concluding remarks

Vasodilation seems to be one of the most sensitive target mechanisms for NO action. The effect occurs in vitro at nanomolar or subnanomolar concentra-

tions. In anesthetized sheep and awake humans, significant lung vasodilator effects of NO at 5 and 10 ppm, respectively, in inhaled air have been observed, and in guinea pigs bronchodilation occurs at 5 ppm. The value 5 ppm can be regarded as established for acute vascular effects in animals, and 10 ppm can be considered the corresponding value for effects in humans. In anesthetized rabbits, significant pulmonary vasodilation is seen at 9 ppm of NO. When, in the same animals, endogenous NO synthesis was inhibited, vasoconstriction occurred. This vasoconstriction was partially counteracted already at 0.15 ppm and was fully reversed at 9 ppm. This finding indicates that endogenous NO can be mimicked by exogenous NO at concentrations commonly found in ambient air. The lowest effect level in individuals with depressed formation of endogenous NO might thus be far lower than in normal individuals, for whom the lowest effect level is likely to be 5 ppm for pulmonary circulation.

It is not clear whether the pulmonary vasodilator effects are to be regarded as adverse or favorable, and NO inhalation is presently being investigated for beneficial effects in patients with derangements in pulmonary function.

Another very sensitive parameter seems to be resistance to infection upon bacterial aerosol exposure, in that an impairment of resistance in female mice has been found at an NO level of 2 ppm. Slight emphysema-like alterations of alveoli occurred at 2 ppm in rats, and thickening of the alveolocapillary membrane was seen in rabbits at 5 ppm. Effects of NO exposure on blood parameters, including leucocytosis, has been found in mice with chronic NO exposure of 2.4 ppm. Methemoglobin formation is seen at 10 ppm of NO and above.

In the future it will be important to consider whether exogenous NO really lowers host resistance, since endogenous NO is important to host defense (7). An alternative explanation might be the rapid generation of NO₂ in NO exposure situations (14). Thus, although NO toxicity data might be biased by concurrent NO₂ generation, the most sensitive parameter seems to be resistance to infection, for which effects have been seen from NO exposure of 2 ppm.

References

- Murad F. Drugs used for the treatment of angina: organic nitrates, calcium-channel blockers, and β -adrenergic antagonists. In: Goodman Gilman A, Rall TW, Nies AS and Taylor P, ed. The pharmacological basis of therapeutics. 80th ed. NY: Pergamon Press, 1990:764-83.
- Noack E, Feelisch M. Molecular mechanisms of nitrovasodilator bioactivation. *Basic Res Cardiol* 1991; 86 suppl 2:37-50.
- Garthwaite J. Glutamate, nitric oxide and cell-cell signalling in the nervous system. *Trends Neurosci* 1991;14: 60-7.
- Snyder SH, Bredt DS. Biological roles of nitric oxide. *Sci Am* 1992;266:68-77.
- Barinaga M. Is nitric oxide the "retrograde messenger"? *Science* 1991;254(5036):1296-7.
- McCall T, Vallance P. Nitric oxide takes centre-stage with newly defined roles. *Trends Pharmacol Sci* 1992;13:1-6.
- Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991;43:109-42.
- Moncada S. The 1991 Ulf v Euler lecture, The L-arginine: nitric oxide pathway. *Acta Physiol Scand* 1992;145:201-27.
- Ignarro LJ. Heme-dependent activation of soluble guanylate cyclase by nitric oxide: regulation of enzyme activity by porphyrins and metalloporphyrins. *Semin Hematol* 1989;26:63-76.
- Hibbs JB Jr, Taintor RR, Vavrin Z, Rachlin EM. Nitric oxide: a cytotoxic activated macrophage effector molecule. *Biochem Biophys Res Commun* 1988;157:87-94.
- Persson MG, Gustafsson LE, Wiklund NP, Moncada S, Hedqvist P. Endogenous nitric oxide as a probable modulator of pulmonary circulation and hypoxic pressor response in vivo. *Acta Physiol Scand* 1990;140:449-57.
- Gustafsson LE, Leone AM, Persson MG, Wiklund NP, Moncada S. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. *Biochem Biophys Res Commun* 1991;181:852-7.
- Frostell C, Blomqvist H, Hedenstierna G, Lundberg J, Zapol WM. Inhaled nitric oxide selectively reverses human hypoxic pulmonary vasoconstriction without causing systemic vasodilation. *Anesthesiology* 1993;78:427-35.
- Foubert L, Fleming B, Latimer R, Jonas M, Oduro A, Borland C, et al. Safety guidelines for use of nitric oxide. *Lancet* 1992;339:1615-16.
- Yoshida K, Kasama K, Kitabake M, Wakabayashi K, Imai M. Changing of nitric oxide in the airway: experiment with model-airway and perfused lung [in Japanese]. *Rep Environ Science* 1981;6:57-61.
- Wagner H-M. Absorption von NO und NO₂ in MIK- und MAK-Konzentrationen bei der Inhalation. *Staub Reinhalt Luft* 1970;30:380-1.
- Yoshida K, Kasama K. Biotransformation of nitric oxide. *Environ Health Perspect* 1987;73: 201-6.
- Vaughan TR Jr, Jennelle LF, Lewis TR. Long-term exposure to low levels of air pollutants: effects on pulmonary function in the beagle. *Arch Environ Health* 1969;19:45-50.
- Greenbaum R, Bay J, Hargreaves MD, Kain ML, Kelman GR, Nunn JF, et al. Effects of higher oxides of nitrogen on the anaesthetized dog. *Br J Anaesth* 1967;39:393-404.
- Pflesser G. Die Bedeutung des Stickstoff-monoxids bei der Vergiftung durch nitroso Gase. *Naunyn Schmiedeberg Arch Exp Pathol Pharmacol* 1935;179:545-57.

21. Oda H, Nogami H, Kusumoto S, Nakajima T, Kurata A, Imai K. Long term exposure to nitric oxide in mice [in Japanese]. *Taiki Osen Kenkyu* 1976;11:150-60.
22. Oda H, Nogami H, Kusumoto S, Nakajima T, Kurata A. Lifetime exposure to 2.4 ppm nitric oxide in mice. *Environ Res* 1980;22:254-63.
23. Murphy SD, Ulrich CE, Frankowitz SH, Xintaras C. Altered function in animals inhaling low concentrations of ozone and nitrogen dioxide. *Am Ind Hyg Assoc J* 1964;25:246-53.
24. Fratacci MD, Frostell CG, Chen TY, Wain JC Jr, Robinson DR, Zapol WM. Inhaled nitric oxide: a selective pulmonary vasodilator of heparin-protamine vasoconstriction in sheep. *Anaesthesiology* 1991;75:990-9.
25. Frostell C, Fratacci MD, Wain JC, Jones R, Zapol WA. Inhaled nitric oxide: a selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. *Circulation* 1991;83:2038-47.
26. von Nieding G. Investigation of the acute effects of nitrogen monoxide on lung function in man. In: VDI-Verlag GmbH. Proceedings of the third international clean air congress, Düsseldorf 1973. Düsseldorf: VDI-Verlag GmbH, 1973:A14-A16.
27. Kagawa J. Respiratory effects of 2-hr exposure to 1 ppm nitric oxide in normal subjects. *Environ Res* 1982;27:485-90.
28. Alving K, Fornhem C, Weitzberg E, Lundberg JM. Nitric oxide mediates cigarette smoke-induced vasodilatory responses in the lung. *Acta Physiol Scand* 1992;146:407-8.
29. Persson MG, Kalzén H, Gustafsson LE. Oxygen or low concentrations of nitric oxide reverse pulmonary vasoconstriction induced by nitric oxide synthesis inhibition in rabbits. *Acta Physiol Scand*. In press.
30. Persson MG, Wiklund NP, Gustafsson LE. Endogenous nitric oxide in single exhalations and the change during exercise. *Am Rev Resp Dis* 1993;148:1210-14.
31. Barnes PJ. Nitric oxide and airways. *Eur Respir J* 1993;6:163-5.
32. Dupuy PM, Shore SA, Drazen JM, Frostell C, Hill WA, Zapol WM. Bronchodilator action of inhaled nitric oxide in guinea pigs. *J Clin Invest* 1992;90:421-8.
33. Högman M, Frostell C, Arnberg H, Hedenstierna G. Inhalation of nitric oxide modulates metacholine-induced bronchoconstriction in the rabbit. *Eur Respir J* 1993;6:177-80.
34. Oda H, Nogami H, Nakajima T. Reaction of hemoglobin with nitric oxide and nitrogen dioxide in mice. *J Toxicol Environ Health* 1980;6:673-8.
35. Azoulay E, Soler P, Blayo MC, Basset F. Nitric oxide effects on lung structure and blood oxygen affinity in rats. *Bull Eur Physiopathol Respir* 1977;13:629-44.
36. Hyde D, Orthoefer J, Dungworth D, Tyler W, Carter R, Lum H. Morphometric and morphologic evaluation of pulmonary lesions in beagle dogs chronically exposed to high ambient levels of air pollutants. *Lab Invest* 1978;38:455-69.
37. Hugod C. Effect of exposure to 43 ppm nitric oxide and 3.6 ppm nitrogen dioxide on rabbit lung: a light and electron microscopic study. *Int Arch Occup Environ Health* 1979;42:159-67.
38. Holt PG, Finlay-Jones LM, Keast D, Papadimitrou JM. Immunological function in mice chronically exposed to nitrogen oxides (NO_x). *Environ Res* 1979;19:154-62.
39. Hugod C. Effect of exposure to 43 ppm nitric oxide and 3.6 ppm nitrogen dioxide on rabbit lung. *Int Arch Occup Environ Health* 1979;42: 159-67.
40. Azoulay E, Bouley G, Blayo MC. Effects of nitric oxide on resistance to bacterial infection in mice. *J Toxicol Environ Health* 1981;7:873-82.
41. Watanabe H, Fukase O, Isomura K. Combined effects of nitrogen oxides and ozone on mice. In: Lee SD, ed. Nitrogen oxides and their effects on health. Ann Arbor, MI: Ann Arbor Science Publishers, 1980:181-9.
42. Katsuki S, Arnold W, Mittal C, Murad F. Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. *J Cyclic Nucleotide Res* 1977;3:23-35.
43. Oda H, Kusumoto S, Nakajima T. Nitrosyl-hemoglobin formation in the blood of animals exposed to nitric oxide. *Arch Environ Health* 1975;30:453-6.
44. Oda H, Nogami H, Nakajima T. Alteration of hemoglobin reacted with nitrogen oxides in vitro. *J Toxicol Sci* 1979;4:299-300.
45. Case GD, Dixon JS, Schooley JC. Interactions of blood metalloproteins with nitrogen oxides and oxidant air pollutants. *Environ Res* 1979;20:43-65.
46. Nakajima T, Oda H, Kusumoto S, Nogami H. Biological effects of nitrogen dioxide and nitric oxide. In: Lee SD, ed. Nitrogen oxides and their effects on health. Ann Arbor, MI: Ann Arbor Science Publishers Inc, 1980:121-41.
47. Chiodi H, Mohler JG. Effects of exposure of blood hemoglobin to nitric oxide. *Environ Res* 1985;37:355-63.
48. Kon K, Maeda N, Shiga T. Effect of nitric oxide on the oxygen transport of human erythrocytes. *J Toxicol Environ Health* 1977;2:1109-13.
49. Sellén H, Winberg P, Gustafsson L, Lundell B, Böök K, Frostell CG. Inhalation of nitric oxide reduced pulmonary hypertension after cardiac surgery in a 3.2 kg infant. *Anaesthesiology* 1993; 78: 577-580.
50. Maeda N, Imaizumi K, Kon K, Shiga T. A kinetic study on functional impairment of nitric oxide-exposed rat erythrocytes. *Environ Health Perspect* 1987;73:171-7.
51. Pepke-Zaba J, Higenbottam TW, Dinh-Xuan AT, Stone D, Wallwork J. Inhaled nitric oxide as a cause of selective pulmonary vasodilatation in pulmonary hypertension. *Lancet* 1991;338: 1173-4.
52. Kinsella JP, Neish SR, Schaffer E, Abam SH. Lowdose inhalational nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet* 1992;340:319-20.
53. Roberts JD, Polander DM, Lang P, Zapol WM. Inhaled nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet* 1992;340: 818-19.
54. Frostell CG, Lönnqvist PA, Sonesson SE, Gustafsson

- LE, Löhr G, Noack G. Near fatal pulmonary hypertension after surgical repair of congenital diaphragmatic hernia: successful use of inhaled nitric oxide. *Anaesthesia* 1993;48:679-83.
55. Pison U, Lopez FA, Heideimeyer CF, Rossaint R, Falke KJ. Inhaled nitric oxide reverses hypoxic pulmonary vasoconstriction without impairing gas exchange. *J Appl Physiol* 1993;74: 1287-92.
56. Weinberg ED. Iron depletion: a defense against intracellular infection and neoplasia. *Life Sci* 1992;50: 1289-97.
57. Reif DW, Simmons RD. Nitric oxide mediates iron release from ferritin. *Arch Biochem Biophys* 1990;283: 537-41.
58. Kanner J, Harel S, Granit R. Nitric oxide as an antioxidant. *Arch Biochem Biophys* 1991;289: 130-6.
59. Kanner J, Harel S, Granit R. Nitric oxide, an inhibitor of lipid oxidation by lipoxygenase, cyclooxygenase and hemoglobin. *JNCI* 1992;84: 827-31.
60. Stadler J, Billiar TR, Curran RD, Stuehr DJ, Ochoa JB, Simmons RL. Effect of exogenous and endogenous nitric oxide on mitochondrial respiration of rat hepatocytes. *Am J Physiol* 1991;260:C910-16.
61. Moriguchi M, Manning LR, Manning JM. Nitric oxide can modify amino acid residues in proteins. *Biochem Biophys Res Commun* 1992;183: 598-604.
62. Wink DA, Kasprzak KS, Maragos CM, Elespuru RK, Misra M, Dunams TM, et al. DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science* 1991;254: 1001-3.
63. Nguyen T, Brunson D, Crespi CL, Penman BW, Wishnok JS, Tannenbaum SR. DNA damage and mutation in human cells exposed to nitric oxide in vitro. *Proc Natl Acad Sci USA* 1992;89:3030-4.
64. Lepoivre M, Fieschi F, Coves J, Thelander L, Fontecave M. Inactivation of ribonucleotide reductase by nitric oxide. *Biochem Biophys Res Commun* 1991;179:442-8.
65. Kwon NS, Stuehr DJ, Nathan CF. Inhibition of tumor cell ribonucleotide reductase by macrophage-derived nitric oxide. *J Exp Med* 1991;174:761-77.
66. Fu Y, Blankenhorn EP. Nitric oxide-induced anti-mitogenic effects in high and low responder rat strains. *J Immunol* 1992;148:2217-22.
67. Garg UC, Hassid A. Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest* 1989;83:1774-7.
68. Nakaki T, Nakayama M, Kato R. Inhibition by nitric oxide and nitric oxide-producing vasodilators of DNA synthesis in vascular smooth muscle cells. *Eur J Pharmacol* 1990; 189:347-53.
69. Bruhne B, Lapetina EG. Activation of a cytosolic ADP-ribosyltransferase by nitric oxide-generating agents. *J Biol Chem* 1989;264:8455-8.
70. Ischiropoulos H, Zhu L, Beckman JS. Peroxy-nitrite formation from macrophage-derived nitric oxide. *Arch Biochem Biophys* 1992;298:446-51.
71. Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite oxidation of sulfhydryls: the cytotoxic potential of superoxide and nitric oxide. *J Biol Chem* 1990;266:4244-50.
72. Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite-induced membrane lipid oxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch Biochem Biophys* 1991;228:481-7.
73. Stamler JS, Simon DI, Osborne JA, Mullins ME, Jaraki O, Michel T, Singel DJ, Loscalzo J. S-nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds. *Proc Natl Acad Sci USA* 1992;89: 444-8.
74. Stamler JS, Singel DJ, Loscalzo J. Biochemistry of nitric oxide and its redox-activated forms. *Science* 1992;258:1898-1902.
75. Ohshima H, Friesen M, Brouet I, Bartsch H. Nitrotyrosine as a new marker for endogenous nitrosation and nitration of proteins. *Food Chem Toxicol* 1990;28(9):647-52.
76. Bartsch H, Shuker DE, Ohshima H. Human nitrosamine exposure: recent dosimetry methods and applications. *Prog Clin Biol Res* 1991;372: 197-204.
77. Martin BL, Wu D, Jakes S, Graves DJ. Chemical influences on the specificity of tyrosine phosphorylation. *J Biol Chem* 1990;265(13): 7108-11.