

block associated with heightened vagal tone. In addition, atropine reverses cholinergically mediated bronchoconstriction and diminishes respiratory tract secretions. These effects contribute to its utility as a preanesthetic medication.

Atropine also decreases gastrointestinal tract motility and secretion. Although various derivatives and congeners of atropine (such as *propantheline*, *isopropamide*, and *glycopyrrolate*) have been advocated in patients with peptic ulcer or with diarrheal syndromes, the chronic use of such agents is limited by other manifestations of parasympathetic inhibition such as dry mouth and urinary retention. The investigational selective  $M_1$  inhibitor pirenzepine inhibits gastric secretion at doses that have minimal anticholinergic effects at other sites; this agent may be useful in the treatment of peptic ulcer. Atropine and its congener *ipratropium*, when given by inhalation, cause bronchodilation and have been used experimentally in the treatment of asthma.

## BIBLIOGRAPHY

- ARNER P: The  $\beta_2$ -adrenergic receptor—a cause and cure of obesity? *N Engl J Med* 333:382, 1995
- CARON MG, LEFKOWITZ RJ: Catecholamine receptors: Structure, function and regulation. *Recent Prog Horm Res* 48:277, 1993
- CHALMERS J, PILOWSKY P: Brainstem and bulbospinal neurotransmitter systems in the control of blood pressure. *J Hypertens* 9:675, 1991
- CLÉMENT K et al: Genetic variation in the  $\beta_2$ -adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. *N Engl J Med* 333:352, 1995
- ESLER M et al: Overflow of catecholamine neurotransmitters to the circulation: Source, fate, and functions. *Physiol Rev* 70:963, 1990
- KUPFERMANN I: Functional studies of cotransmission. *Physiol Rev* 71(3):683, 1991
- LANDSBERG L, YOUNG JB: Catecholamines and the adrenal medulla, in *Williams' Textbook of Endocrinology*, 8th ed, DW Foster, JD Wilson (eds). Philadelphia, Saunders, 1992, p 621
- LOKHANDWALA MF, AMENTA F: Anatomical distribution and function of dopamine receptors in the kidney. *FASEB J* 5:3023, 1991
- LOW PA: Autonomic nervous system function. *J Clin Neurophysiol* 10:14, 1993
- MEISTER B, APERIA A: Molecular mechanisms involved in catecholamine regulation of sodium transport. *Semin Nephrol* 13:41, 1993
- NELSON H:  $\beta$ -Adrenergic bronchodilators. *N Engl J Med* 333:499, 1995
- PACHOLCZYK T et al: Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter. *Nature* 350:350, 1991
- RUFFOLO RR et al: Structure and function of  $\alpha$ -adrenoceptors. *Pharmacol Rev* 43:475, 1991
- VAN ZWIETEN PA et al: The parasympathetic system and its muscarinic receptors in hypertensive disease. *J Hypertens* 13:1079, 1995
- WIDÉN E et al: Association of a polymorphism in the  $\beta_2$ -adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. *N Engl J Med* 333:348, 1995
- WILLIAMS JL et al: Area postrema: A unique regulator of cardiovascular function. *News Physiol Sci* 7:30, 1992

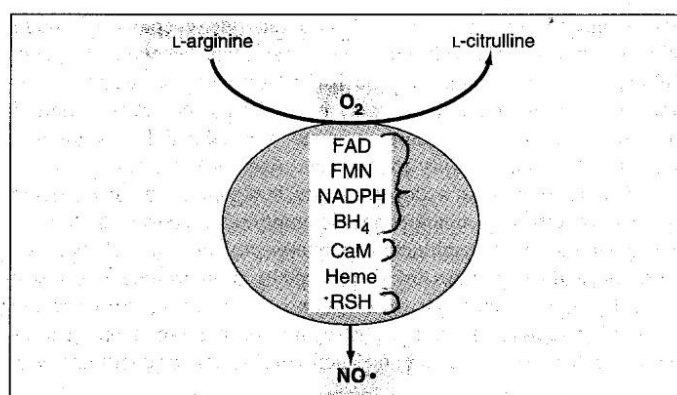
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Joseph Loscalzo

## NITRIC OXIDE: BIOLOGIC AND MEDICAL IMPLICATIONS

Nitric oxide ( $\text{NO}$ ) is a simple, heterodiatomic molecule with broad and diverse effects in human biology that have been recognized only recently. In 1980, Furchgott and Zawadzki reported that a product of the endothelial cell causes vasorelaxation, and this endothelium-derived relaxing factor (EDRF) was eventually shown to be  $\text{NO}$ .  $\text{NO}$  is now known to be produced by many cell types and to exert a wide range of biologic effects.

$\text{NO}$  is synthesized by a family of enzymes known as the nitric oxide synthases (NOSs) (Fig. 71-1). Three distinct isoforms have been identified, of which two are named after the cell types from which



**FIGURE 71-1** Nitric oxide synthases catalyze the five-electron oxidation of L-arginine to L-citrulline and nitric oxide. Cofactor requirements include flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), reduced  $\beta$ -nicotinamide adenine dinucleotide phosphate (NADPH), tetrahydrobiopterin (BH<sub>4</sub>), calcium-calmodulin (CaM), a heme complex, and a thiol equivalent (RSH). (Adapted, with permission, from the Annual Review of Pharmacology and Toxicology, vol. 35, 1995, by Annual Reviews, Inc.)

they were first cloned: neuronal NOS (*nNOS*, *Nos1* gene product); inducible NOS (*iNOS*, *Nos2* gene product), present in monocytes/macrophages, smooth muscle cells, microvascular endothelial cells, fibroblasts, cardiomyocytes, hepatocytes, and megakaryocytes; and endothelial NOS (*eNOS*, *Nos3* gene product). As a free radical,  $\text{NO}$  readily undergoes addition, substitution, redox, and chain-terminating reactions, which serve as the molecular basis for its biologic effects.  $\text{NO}$  reacts with heme-bound iron to form a nitrosylated adduct. Reaction with the prosthetic heme group of guanylyl cyclase activates the enzyme; reaction with the heme group of hemoglobin traps  $\text{NO}$ , making it biologically inactive and converting ferrous to ferric hemoglobin; reaction with the prosthetic heme groups of cytochromes uncouples oxidative phosphorylation.

**PHYSIOLOGIC ACTIONS**  $\text{NO}$  is implicated in a wide variety of physiologic effects. The reaction of  $\text{NO}$  with the heme group of guanylyl cyclase is the principal effector reaction of  $\text{NO}$  in the cardiovascular system. This reaction leads to activation of guanylyl cyclase. In smooth muscle cells, the increase in cyclic guanosine 5'-monophosphate (cyclic GMP) leads to activation of cyclic GMP-dependent protein kinase, which, in turn, phosphorylates myosin light chain kinase. Phosphorylated myosin light chain kinase has a reduced affinity for the calcium-calmodulin complex and is less efficient at phosphorylating myosin light chain. Failure to phosphorylate the regulatory light chains of myosin stabilizes the inactive form of the enzyme and reduces smooth muscle contraction, thereby reducing vascular tone. Agonist-mediated increases in endothelial cell calcium activate *eNOS*. In addition, flow-mediated vasodilation with exercise depends in part on the flow-sensitive increase in endothelial cell calcium, which leads to an increase in *eNOS* activity.

Similar effects are exerted by  $\text{NO}$  in smooth muscle cells of other organ systems.  $\text{NO}$  relaxes gastrointestinal smooth muscle and leads to reduced motility, relaxation of the sphincter of Oddi, and relaxation of the lower esophageal sphincter. Relaxation of bronchial smooth muscle can be provoked by inhaled  $\text{NO}$ , and endogenously produced  $\text{NO}$  may contribute to the maintenance of basal bronchial and basal pulmonary arterial tone, as well.

Other effects of *eNOS* include maintenance of vascular integrity, impairment of leukocyte adhesion to the endothelium, and inhibition of smooth muscle migration and proliferation. Endothelial  $\text{NO}$  also plays a critical role in hemostasis, making an important contribution to the normal inhibition of platelet function. Basal production of  $\text{NO}$  by *eNOS* inhibits both adhesion and aggregation of platelets in the vasculature. Inhibition of platelet adhesion is a property of  $\text{NO}$  not shared by the other principal antiplatelet product of the endothelium, prostacyclin.

Endothelial  $\text{NO}$  is an important determinant of cerebral blood flow. *nNOS* in neuronal and glial cells contributes to the regulation

of cerebrovascular tone and to memory and learning through its involvement in long-term potentiation in the central nervous system. NO• is a likely transmitter of nonadrenergic, noncholinergic neurons and may thereby participate in the regulation of myocardial contractility, heart rate, gastrointestinal motility, bronchial tone, and penile erection.

The production of NO• by *iNOS* in macrophages, lymphocytes, and neutrophils is an important determinant of immune and inflammatory responses. The bactericidal, fungicidal, viricidal, parasiticidal, and tumoricidal activities of macrophages are determined in part by the robust elaboration of NO• by *iNOS*. NO• also limits lymphocyte proliferation and attenuates the allogeneic immune response. Given the broad range of non-immune cell types shown to express *iNOS*, some investigators believe that NO• produced by this isoform is involved in nonspecific immunity, especially in the lung and liver. By a similar mechanism, NO• produced by *iNOS* may also be involved in apoptotic responses in a variety of cell types.

**PATHOPHYSIOLOGIC EFFECTS OF NITRIC OXIDE** Both a deficiency and an excess of NO• are believed to be involved in several pathophysiologic states. NO• is a critical determinant of basal vascular tone, and a deficiency of NO• is associated with *hypertension*, as illustrated by recent observations in a murine model of *Nos3* gene inactivation by homologous recombination. Nevertheless, genetic linkage studies have thus far failed to suggest an association between the *Nos3* locus and essential hypertension. However, Deng and Rapp have demonstrated a clear linkage between the *Nos2* locus and salt-sensitive hypertension in the Dahl S rat. Also, the provision of L-arginine in the diet of these animals corrects the hypertensive response to dietary sodium chloride. In chronic renal failure, the plasma concentration of dimethylarginine, a naturally occurring derivative of L-arginine, is increased and competitively inhibits nitric oxide synthase activity, possibly contributing to the hypertension of chronic renal failure.

Common disorders that promote atherosclerosis, such as hypertension, hyperlipidemia, smoking, and diabetes, are all associated with abnormal endothelial function, one manifestation of which is a comparative deficiency of bioactive NO•. This deficiency, which represents either a true deficiency of the molecule or inactivation of it by reactive, oxygen-derived free radicals, is accompanied by increased vascular tone, reduced antithrombotic activity, decreased antiproliferative action, increased endothelial permeability, and enhanced susceptibility of low-density lipoprotein to oxidation. These many interrelated actions may promote atherosclerosis and its complications.

Expression of *iNOS* occurs in several disease states, the most prominent being *bacterial sepsis*. Induction of *Nos2* is induced by endotoxin and cytokines, and the elaboration of NO• in this setting accounts for the hypotension of septic shock states and contributes to the hemorrhagic diathesis of sepsis by profoundly inhibiting platelet function. The myocardial depression associated with septic shock may be explained in part by the inhibition of myocardial contractility by NO•. The cytokine-rich milieu that accompanies ischemia-reperfusion injury may also contribute to increased elaboration of NO• through increased expression of *iNOS* and the accompanying myocardial depression that follows successful coronary thrombolysis or revascularization.

A deficiency of NO•-producing neurons in the gastrointestinal tract is believed to be responsible for certain abnormalities in gastrointestinal motility, such as *Hirschsprung's disease*, *achalasia*, and *chronic intestinal pseudo-obstruction*. NO• is also believed to play an important role in gastric cytoprotection, possibly by way of increased mucosal blood flow and the modulation of gastric epithelial function.

Increased NO• production owing to the induction of *Nos2* in hepatocytes, fibroblasts, and endothelial cells is believed to underlie the hyperdynamic circulatory state of Laennec's cirrhosis. Also, NO• produced by hepatocyte *iNOS* plays a role in protection of these cells against a variety of hepatic toxins, including ethanol and acetaminophen. NO• inhibits protein synthesis in the hepatocyte at the posttranslational level and inhibits several mitochondrial enzymes involved in

electron transport, including *cis*-aconitase, NADH-ubiquinone oxidoreductase, and succinate-ubiquinone oxidoreductase. NO• also inhibits glyceraldehyde-3-phosphate dehydrogenase activity in hepatocytes, thereby influencing glucose metabolism. On exposure to an oxidizing hepatic toxin, NO• prevents the consumption of cell reducing equivalents (reduced glutathione). These several effects of NO• on hepatic function suggest that hepatoprotection results from a reduction in the metabolic activity of the cell, which conserves the cell's energy stores.

Cytotoxicity by NO• also appears to be important in the central nervous system. Following neuronal injury, large amounts of L-glutamate are released and elicit neurotoxicity by activation of N-methyl-D-aspartate receptors. Activation of these receptors augments *nNOS* activity. While *nNOS*-containing neurons are resistant to the cytotoxic effects of receptor stimulation, NO• released from them is responsible for cytotoxicity in adjacent neurons. Interestingly, NO• may also be cytoprotective in the central nervous system, an effect that depends on the molecule's redox state: NO<sup>+</sup> reacts with the N-methyl-D-aspartate receptor and thereby limits entry of excessive Ca<sup>2+</sup> into the cell cytosol; NO•, by contrast, promotes Ca<sup>2+</sup> entry into the cell and thus cytotoxicity.

Impairment of NO• production by endothelial cells in disorders of endothelial dysfunction or following percutaneous angioplasty may help to facilitate smooth muscle proliferative responses in these settings. In addition, elevated NO• may contribute to cytotoxic mechanisms in *graft-versus-host disease* and in *transplant rejection*. The cytoprotective and cytotoxic effects of NO• are important and contrasting; the balance between them is determined by the local concentration of NO• in a given organ, the availability of other free radicals that can react with NO• to form other oxidative compounds (such as O<sub>2</sub>•, which reacts with NO• to form OONO•), and the susceptibility of the cell or organ to the toxic effects of oxidant stress.

**THERAPEUTIC IMPLICATIONS** Therapeutic manipulation of NO• levels—by providing the compound or by inhibiting its production—has profound effects in many clinical settings. For over 100 years, congeners of NO•, the nitrovasodilators, have been used to provide exogenous NO• to dysfunctional coronary arteries. These agents, including nitroglycerin, isosorbide mononitrate and dinitrate, and nitroprusside, promote vasodilation and platelet inhibition and are metabolized to NO• (or NO<sup>+</sup>) to achieve these effects. Inhibition of *iNOS* can be used to restore normal arterial pressures in the setting of *septic shock*; however, the risk of intravascular platelet thrombosis is increased by this approach in animals. Interestingly, glucocorticoids inhibit *Nos2* transcription, which may account for part of their protective effect in septic syndromes. Owing to the importance of NO• in penile erection, NO• donors may be useful for the treatment of *erectile impotence*.

Given the relative pulmonary selectivity of inhaled NO•, this gas may be useful at concentration of 10 to 40 ppm for the treatment of *persistent pulmonary hypertension of the newborn*, the pulmonary vasoconstriction that accompanies congenital diaphragmatic hernia, *primary pulmonary hypertension*, and *adult respiratory distress syndrome*. Higher concentration of inhaled NO• may be toxic, owing to the reaction of NO• with oxygen to produce NO<sub>2</sub>.

NO• may also be useful in the inhibition of proliferative responses following vascular injury. Local delivery of a long-acting NO<sup>+</sup> donor or gene therapy with *Nos3* limits vascular smooth muscle proliferation following denuding endothelial injury, which suggests potentially useful therapies for limiting restenosis following angioplasty.

The simplicity of the NO• molecule belies its wide range of complex biologic, pathobiologic, and therapeutic effects. Our understanding of the biologic roles of this molecule and its potential therapeutic uses are in their infancy. With a clearer understanding of the organ-specific effects of NO•, its toxic and therapeutic potential in specific organ systems, and the mechanisms by which its actions may be inhibited or promoted, the full potential of this molecule as a therapeutic agent may be realized.