Nitric Oxide and Cardiovascular Dysfunction

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Prior to the mid-1980s, nitric oxide (NO) was viewed as an environmental pollutant but not as a compound of physiological significance. Thus, it was a skeptical audience that first heard the pronouncement at a scientific meeting in 1986, that NO was the identity of the elusive endothelium-derived relaxing factor,[1,2] a mediator of vasorelaxation in response to numerous endogenous stimuli.[3] Since then, the simple gas NO has gone from obscurity to center stage, being identified as a key player in physiologic processes as diverse as blood pressure maintenance, neural transmission, and immunologic defense.[4] In addition to its physiological roles, NO has been implicated in the pathogenesis of a multitude of disease states,[5] many of which are of primary interest to the cardiovascular surgeon: circulatory shock, atherosclerosis, diabetes mellitus, and ischemia-reperfusion injury. Recent years has seen NO biology emerge as an exciting and extremely fertile area of biomedical investigation. To fully understand the molecular basis of many clinical problems facing the cardiovascular surgeon, appreciation of NO's involvement is essential.

NITRIC OXIDE IN VASCULAR PHYSIOLOGY

NO is a potent vasorelaxant which plays a fundamental physiological role in vascular homeostasis and blood pressure regulation. Relaxation of vascular smooth muscle by NO is attributed to reaction with ferrous-heme in the soluble isoform of guanylyl cyclase (sGC); this results in activation of sGC and intracellular accumulation of cyclic guanosine monophosphate (cGMP).[6] cGMP acts via incompletely understood mechanisms to elicit a reduction in free intracellular calcium, thereby dampening vascular tone (Fig. 1). Therapeutic vasorelaxants such as sodium nitroprusside and nitroglycerine similarly act by transferring their NO moieties to heme in sGC, resulting in enzyme activation and increased cGMP. While these medications have been in use for many years, our recent appreciation of NO's physiological role

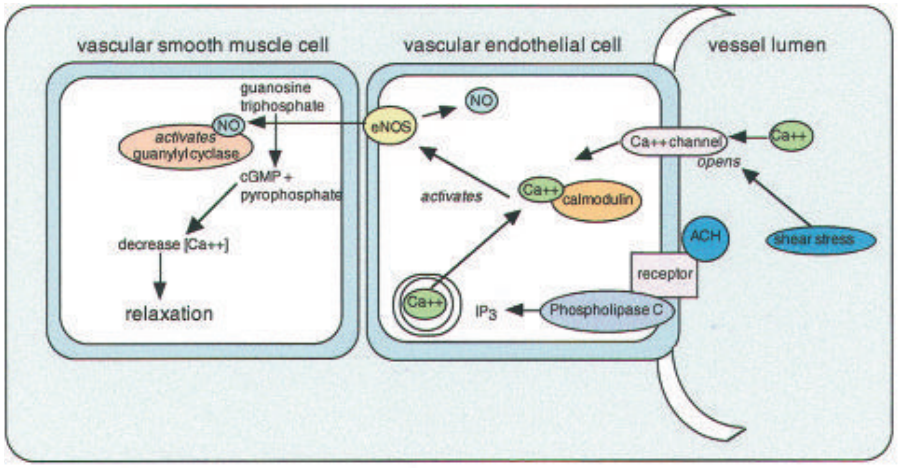

Figure 1. Proposed mechanism by which nitric oxide (NO) relaxes vascular smooth muscle and produces vasodilatation. Abbreviations: cGMP, cyclic guanosine monophosphate; eNOS, endothelial nitric oxide synthase; ACH, acetylcholine; IP3, inositol 1,4,5-triphosphate.

in vasodilatation prompts us to view their actions in a new light, as NO replacement therapy.

A schematic which depicts the role of NO in vasoregulation is provided in Figure 1. Many endogenous vasodilatory agents (e.g., acetylcholine [ACH], bradykinin [BK], ADP, ATP, histamine, substance P, leukotrienes), as well as shear-stress arising from pulsatile blood flow, cause smooth muscle relaxation in vivo, secondary to the synthesis and release of endothelial-derived NO.[7,8] Following binding to their cognate endothelial cell surface receptors and acting through a G-protein, vasodilators such as ACH and BK stimulate membrane-bound phospholipase C. This leads to the release of inositol 1,4,5-triphosphate and diacylglycerol which, in turn, cause the release of Ca^{++} from intracellular stores. Shear-stress also elevates intracellular calcium in endothelial cells, apparently via the opening of cell membrane-bound potassium channels.[9] These transient elevations in basal levels of intracellular Ca^{++} (from 50 nM to 1,000 nM) result in formation of Ca^{++}/calmodulin complexes which bind to and activate the endothelial isoform of nitric oxide synthase (eNOS). The net result is pulsatile NO release coincident with intracellular Ca^{++} transients. NO then relaxes the underlying vascular smooth muscle via the cGMP system, as outlined above. Release of NO from nerve terminals may also contribute to the regulation of vascular tone, either directly[10] or through centrally mediated cardiovascular reflexes.[11]

NITRIC OXIDE PRODUCTION AND THE NITRIC OXIDE SYNTHASES

Nitric oxide synthases (NOS) arise from three distinct genes whose products are approximately 50% homologous with one another at the amino acid level.[12] Although two of the NOS isoforms are named for the anatomic sites where they were first identified, that is, endothelial cells (eNOS, Fig. 2a) and neurons (nNOS, Fig. 2B), it is now appreciated that localization of these isoforms is not restricted to the originally identified tissues. For example, nNOS has been identified on skeletal muscle cell membranes[13] and eNOS can be found in certain neuronal[14] and epithelial cells.[15] Since they are continuously expressed in "normal" tissues, eNOS and nNOS are referred to as constitutive NOS isoforms. The third isoform is inducible NOS (iNOS), which appears in macrophages and various other cell types (including vascular cells) only after exposure to immunostimulants such as interferon-g, tumor necrosis factor, interleukins-1 and -2, and bacterial lipopolysaccharides (LPS).[12] Inducible NOS differs from constitutive NOS isoforms in that even at low basal levels of intracellular Ca^{++}, it retains bound calmodulin and is active.[16] Thus, iNOS activity is Ca^{++}-independent and may produces large quantities of NO continuously until substrates are exhausted.

NITRIC OXIDE SYNTHASE FUNCTION

The three NOS enzymes are homodimeric proteins which catalyze two suc-

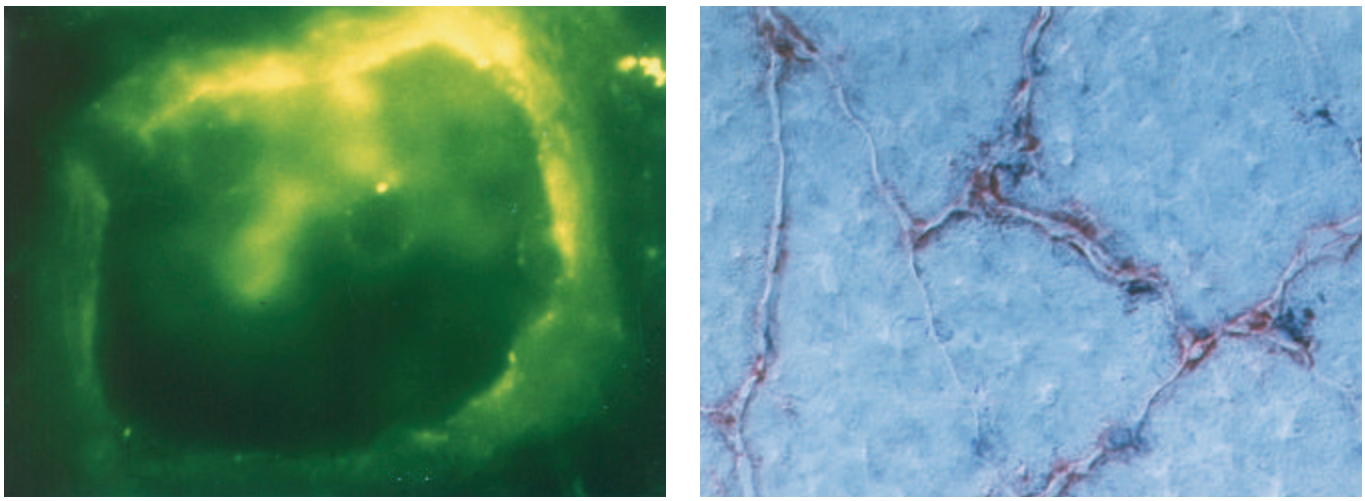

Figure 2. Rat hind limb skeletal muscle subjected to high-grade partial ischemia for two hours, followed by one hour of reperfusion, in vivo. (a) Polyclonal antibodies to the endothelial isoform of nitric oxide synthase (eNOS) highlight the endothelial border of a blood vessel, as demonstrated by immunofluorescent staining. (b) Monoclonal antibodies to the neuronal isoform of nitric oxide synthase (nNOS) demonstrates the presence of the enzyme in apparent association with the cell membrane, as evidenced by ABC-alkaline phosphatase staining.

cessive mono-oxygenation reactions that result in a 5 electron oxidation of one of the equivalent guanidino nitrogens in L-arginine; products are NO and L-citrulline (Fig. 3). This complex reaction occurs at the expense of 2 moles of molecular oxygen, 1.5 moles of NADPH and involves four distinct prosthetic groups: flavin-adenine dinucleotide (FAD), flavin mononucleotide (FMN), tetrahydrobiopterin (BH4), and iron protoporphyrin IX (heme). While each of these cofactors is widely found in nature, NOS is the only enzyme known to require all four.[17] Ca^{++}/calmodulin serves to gate the flux of NADPH-derived electrons, one at a time, from the C-terminal reductase domain of NOSs to the heme-iron in the N-terminal oxygenase domain.[18] Analogous to the cytochrome P-450 mixed function oxygenases, heme serves as the site of activation of molecular oxygen for reaction with substrate.[19] N^{ω}-Hydroxy-L-arginine is the product of the initial monoxygenation step catalyzed by NOS, and is an isolatable intermediate.[20]

NO IN CARDIOVASCULAR PATHOPHYSIOLOGY

Blood Pressure Regulation

The constitutive enzyme eNOS continually synthesizes "puffs" of NO to exert its important physiologic function of regulating blood vessel tone and patency. Normal blood pressure maintenance requires endothelial production of NO to oppose the vasoconstrictory influence of the sympathetic nervous system. Accordingly, animal studies have demonstrated that when eNOS is inhibited pharmacologically, blood pressure in experimental animals increases by as much as 40%.[21,50] Tonic NO production in human blood vessels in vivo has similarly been observed.[22] Diseases that interfere with endothelial function, such as atherosclerosis, decrease levels of bioactive NO, cause vasoconstriction, and contribute to hypertension.[23] On the other hand, excess NO production can lead to profound hypotension and cardiovacular collapse, such as that seen in patients with severe septic shock (see below).

Platelet Function

In blood vessels, NO freely diffuses from endothelial cells into platelets, where it serves to inhibit platelet adhesion to the endothelium and platelet binding to other platelets (platelet aggregation).[24] NO is also produced by platelets themselves, where it serves in an autocrine manner to limit endothelial adhesion[25] and aggregation.[26] Interestingly, the antithrombotic effect of prostacyclin, another important endogenous inhibitor of platelet aggregation, synergizes with that of NO.[27] Thus, constitutive NO production plays a protective role to prevent thrombosis.

Atherosclerosis

A large body of evidence demonstrates that atherosclerotic vessels from animal models and man have a deficit in their capacity to produce vasorelaxant NO.[28] While this was originally thought to reflect decreased eNOS activity in atherosclerotic vessels, the more significant factor may be a decrease in the bioactive lifetime of NO, due to enhanced reaction with oxygen-derived free radicals.[23] Nonetheless, recent evidence suggests that oxidized low-density lipoprotein (LDL) inhibits eNOS protein synthesis directly, via suppression of both eNOS gene transcription and mRNA destabilization.[29] As smooth muscle cell (SMC) proliferation is inhibited by NO,[30] impaired endothelial NO synthesis could also serve to trigger intimal hyperplasia in the atherosclerotic lesion. The atherogenic effect of LDL may therefore be in part mediated by inhibition of NO. Production of NO within the atherosclerotic lesion and reaction with superoxide radical (0_2•) has been confirmed by Beckman and colleagues.[31] NO reacts rapidly with 0_2• to form peroxynitrite ($ONOO^-$), a potent oxidant which readily attacks cell membrane lipids and cellular proteins.[32] A marker of ONOO- attack is nitration of tyrosine residues on proteins. Antibodies specific for nitrated tyrosine have revealed extensive antibody binding within human coronary artery atherosclerotic plaques and early subintimal fatty streaks.[31] This striking finding supports the view that loss of NO via reaction with oxygen-derived species serves as a potential source of harmful free radical oxidants that may be central to LDL oxidation, and perhaps initiation or propagation of atherosclerosis.[33]

While constitutive NO production would tend to prevent atherogenesis via suppression of platelet adhesion and smooth muscle cell proliferation, NO overproduction could also result in oxidative species which promote atherogenesis. A recent clinical investigation of the relationship between cigarette smoking and oxidant activity found that smokers had higher plasma levels of F_2isoprostanes, products of the free radical-catalyzed peroxidation of arachidonic acid.[34] Cigarette smoke contains significant amounts of NO and NO-derived oxidants which could trigger arachidonate peroxidation. The promotion of blood vessel disease by smoking is likely to occur in part from induction of oxidative stress, with NO and derived species as contributors.

Ischemia-Reperfusion Injury

It is a well-known and extensively studied phenomenon that tissue necrosis occurs upon reoxygenation of previously ischemic tissues.[35,36] Clinical conditions in which ischemia may be followed by reperfusion include patients undergoing extracorporeal circulation during cardiac surgery, thrombolytic therapy or angioplasty to re-establish acute vascular occlusion in coronary or extremity vessels, organ transplantation, and traumatic extremity amputa-

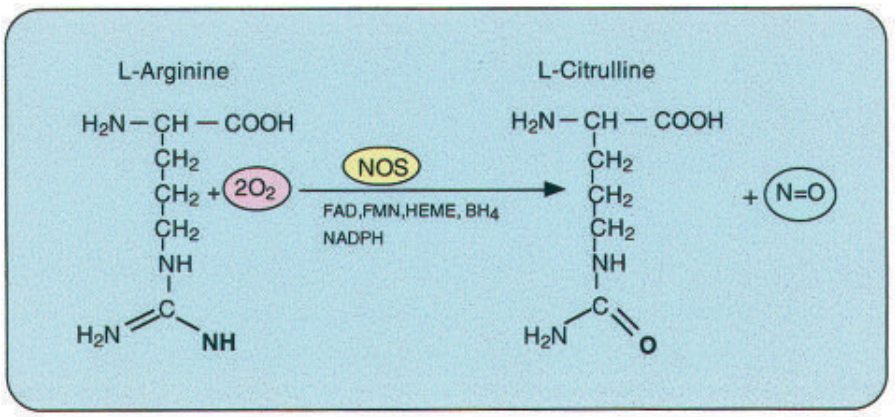

Figure 3. Mechanism of nitric oxide (NO) synthesis. NOS = nitric oxide synthase; FAD = flavin-adenine dinucleotide; FMN = flavin mononucleotide; HEME = iron protoporphyrin IX; BH4 = tetrahydrobiopterin.

tion and replantation. Post-ischemic tissue injury is mediated by oxygen-derived free radicals.[37] One prominent source of free radicals in ischemia is the ubiquitous cellular enzyme xanthine dehydrogenase (XD), which serves in the breakdown of adenine-based nucleic acids. Under ischemic conditions, XD is converted to xanthine oxidase (XO). The enzyme XO produces superoxide radical from the breakdown of hypoxanthine upon reoxygenation.[38]

Initial studies suggest that NO may protect against ischemia-reperfusion injury by acting as a radical scavenger or by downregulating xanthine oxidase function.[39] Indeed, studies with feline hearts have shown reduction of infarct size with administration of NO donating drugs.[40] Direct evidence has been provided for increased NOS activity with ischemia-reperfusion of cardiac muscle and significant protection against injury is afforded by prophylaxis with a NOS inhibition.[41]

NO may also have a cytotoxic effect during ischemia-reperfusion. During prolonged ischemia, intracellular calcium rises as its energy-dependent sequestration decreases due to diminished mitochondrial respiration and depletion of cellular ATP. Since the constitutive NOS enzymes (eNOS and nNOS) are calcium-dependent, as Beckman et al. point out, NOS activity would predictably be upregulated during ischemia.[32] Despite an enhanced NOS activity, however, NO production during ischemia should be low due to limited availability of oxygen, a required NOS substrate. Nonetheless, upon reperfusion, restored oxygen tension in the face of enhanced intracellular Ca^{++} would predictably cause synthesis of excessive levels of NO. In this setting, NO would avidly react with superoxide anion that is concomitantly produced during reperfusion, to yield large quantities of the potent oxidant peroxynitrite. Peroxynitrite reacts with cell membrane lipids and protein targets with significant potential for tissue injury.[42]

Our laboratory is currently investigating whether nNOS, present in skeletal muscle cell membranes (Fig. 2b), contributes to ischemia-reperfusion injury via this proposed peroxynitrite pathway. Rat skeletal muscle subjected to two hours of ischemia and one hour of reperfusion by infrarenal aortic cross clamping, shows accumulation of protein nitrotyrosine residues (Fig. 4), a "fingerprint" for peroxynitrite attack on protein. This finding therefore supports the view that NO-derived peroxynitrite may be an important participant in ischemia-reperfusion injury of skeletal muscle.

Bypass and Transplant Surgery

NO is involved in other processes relevant to cardiovascular surgery, such as coronary bypass grafting. Production of NO by arterial versus venous endothelium may contribute to the superior patency rates of certain arterial grafts as compared to venous grafts.[43] Moreover, excessive NO production has been associated with organ rejection and graft versus host disease.[44,45] Importantly, blocking NO synthesis in animals has been found to prolong graft function and survival[46] and therefore may have future utility in the clinic. Experimental and clinical application of NO to treatment of pulmonary hypertension suggests potential utility of NO replacement therapy for this condition.[47,48]

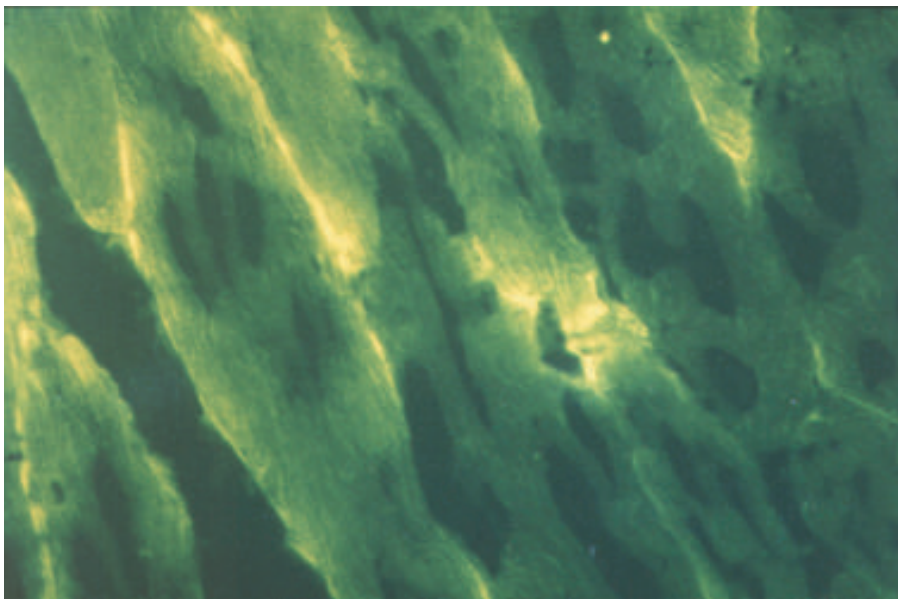

Figure 4. Rat hind limb skeletal muscle subjected to high-grade partial ischemia for two hours, followed by one hour of reperfusion, in vivo. Nitrotyrosine, the reaction product of peroxynitrite (ONOO-) with skeletal muscle protein tyrosine residues, is visualized by epifluorescence using a specific mouse monoclonal antibody.

Immunostimulant-induced Vascular Shock

NO overproduction by iNOS is responsible for septic and cytokine-induced hypotension[49,50] and the characteristic resistance to vasoconstrictors seen in these patients.[8] This view has recently been confirmed in studies of iNOS gene-targeted "knockout" mice, which do not become hypotensive and are not killed by a dose of LPS that is lethal to 100% of $iNOS^{+/+}$ mice.[51] Thus, the use of NOS inhibitors for treating septic shock is an active area of clinical investigation and shows promise for improved therapy.[52] Nonetheless, use of NOS inhibitors to treat sepsis is tempered by the knowledge that iNOS-selective inhibitors will be needed to minimize side effects arising from eNOS or nNOS inhibition. Clinical use of NOS inhibitors is also handicapped by the finding in rodents that iNOS-derived NO is important for macrophage-mediated cytostasis of many bacteria. On a positive note, however, the participation of NO as a mediator of host-defense reactions in man is significantly less than for rodents, if it functions at all.[53] Mechanisms by which NO acts as a mediator of cytostasis by macrophages involve the inactivation of vital iron-containing enzymes; pivotal among these are mitochondrial complexes I and II, aconitase and ribonucleotide reductase.[5] Similarly leukocytes generate nitric oxide-derived free radicals, including peroxynitrite, which are used in host defense against invading microbes.

SUMMARY

NO is considered to be a "double-edged sword," where production is essential for host-defense and many physiologic processes, while overpro-

duction can be toxic. Understanding this dual role of NO lends hope to the prospect of developing new therapeutic interventions aimed at preventing diseases associated with dysregulation of NO synthesis. While NO donors may be useful in treating conditions manifest by too little NO (e.g., hypertension), NO inhibitors, such as the arginine analog N^{ω}methylL-arginine, may be useful in alleviating NO excess (e.g., septic shock). The possibility of clinically manipulating NO synthesis offers the surgeon abundant opportunities for improved patient management. STI

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