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EDRF/Nitric Oxide

The Endogenous Nitrovasodilator and a New Cellular Messenger

Anesthesiologists have been using sodium nitroprusside, nitroglycerin, and other nitrovasodilators in their clinical practice for more than two decades. The action of all of these drugs is mediated by their common metabolism or spontaneous reduction to nitric oxide (NO).[1] Recently, an "endogenous nitrovasodilator" has been discovered[2] which promises to be of enormous physiologic, pathophysiologic, and clinical significance. This substance, endothelium-derived relaxing factor (EDRF), appears to be NO itself or a similar NO-containing species such as a nitrosothiol. First reported in vascular endothelium,[2] where it serves as a potent determinant of basal and induced vasodilator tone, EDRF/NO was found to be a labile, diffusible factor released from the endothelium under basal conditions and in response to a wide variety of endogenous hormones and chemicals that increase intracellular calcium (*e.g.*, adenosine triphosphate, adenosine diphosphate, bradykinin, histamine, and thrombin). The increase in calcium stimulates the production of EDRF/NO from the amino acid L-arginine by EDRF/NO synthase, a constitutive enzyme that is calcium-, calmodulin-, and nicotinamide adenine dinucleotide phosphate-dependent. Once produced, EDRF/NO diffuses to the vascular smooth muscle, where it activates soluble guanylate cyclase, resulting in the production of cyclic guanosine monophosphate (GMP) from guanosine triphosphate, which in turn leads to vasodilation. (For review, see reference 3 and footnote.*)

The implications of EDRF/NO go well beyond its actions in the endothelial cell and blood vessels. This L-arginine-to-NO pathway appears to be the transduction mechanism for the activation of soluble guanylate cyclase in all cells in which the cyclase is present.[3] It is a newly discovered mechanism by which cells regulate their own function and influence that of others. This cell messenger function of EDRF/NO has been most extensively studied in the vascular, immune, and nervous systems. In the vasculature it is a potent vasodilator that also inhibits platelet aggregation and adhesion.[3,*] In the immune system, it is an effector mechanism for macrophage-induced cytotoxicity,[4,5] whereas in the nervous system it is a neurotransmitter in several specific neural pathways.[6,7]

Although there is now extensive literature on the physiologic and pathophysiologic implications of EDRF/NO, Fratacci *et al.*,[8] in this issue of ANESTHESIOLOGY, present one of the first therapeutic applications of these exciting and promising new research findings. They describe the novel use of inhaled low concentrations of NO to selectively vasodilate the pulmonary vascular bed and to reverse heparin–protamine vasoconstriction in sheep. This, along with the initial report by these authors in *Circulation*,[9] is a landmark finding in pulmonary vascular research. Pulmonary hypertension is a hemodynamic abnormality that is shared by a variety of acute and chronic pulmonary disease states. Regardless of the etiology of pulmonary hypertension, the increased pressure in the pulmonary circulation results in a progressive inability of

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the right ventricle to sustain its output and frequently leads to right ventricular failure and death. Interventions that reduce right ventricular afterload, thereby improving right ventricular function, have long been sought, and many different vasodilator agents have been used in an attempt to achieve such a goal. The optimal vasodilator would selectively dilate the pulmonary vasculature without altering systemic vascular resistance. Unfortunately, no selective or even highly preferential pulmonary vasodilator agent—until now—had been identified.

Inhaled NO may prove to be of tremendous clinical benefit in the management of the acute pulmonary hypertension associated with a wide variety of disease states, including adult respiratory distress syndrome and persistent pulmonary hypertension of the newborn, as well as in the intraoperative management of patients with elevated pulmonary vascular resistance. One particularly notable situation would be the child with congenital heart disease and pulmonary hypertension undergoing surgical repair. It is often impossible to separate such children from cardiopulmonary bypass because of right ventricular failure due to the elevated pulmonary vascular resistance. Administration of traditional vasodilators in this situation often results in disastrous systemic hypotension. A selective pulmonary vasodilator administered in the perioperative period would be of tremendous advantage.

Nevertheless, in approaching the therapeutic application of inhaled NO, its potential for serious, even lethal, toxicity must be given due consideration. First known as a toxin and air pollutant, NO is the etiologic agent in the pulmonary complications of silo filler's disease. Although high concentrations of NO (greater than 5,000 ppm) have proven lethal in animal studies,[10] the concentrations of NO required for selective pulmonary vasodilation (180 ppm and less) appeared to be without direct toxic effects when administered for short periods of time. Consideration must be given to cumulative direct cytotoxic effects of NO with prolonged administration as well as to the effect of increased plasma concentrations of nitrites, nitrates, and the toxic nitrogen dioxide gas—all products of the reaction of NO with oxygen.

NO binds to the iron of heme-based proteins and thus is avidly bound and inactivated by hemoglobin. The short half-life of NO in combination with its rapid inactivation by hemoglobin is likely to account for the pulmonary vascular selectivity observed. It is ironic that this interaction with hemoglobin, while providing pulmonary selectivity, may limit the use of NO to anything but the more acute and transient forms of pulmonary hypertension. As discussed by Fratacci *et al.*,[8] nitrosylhemoglobin is oxidized to methemoglobin, which accumulates during the course of NO inhalation. It is also extremely important to realize that NO may bind not just to hemoglobin and guanylate cyclase, but to any heme-based enzyme. Indeed, inactivation of cytochromes required for electron transport[11] and of other iron-centered heme- and non–heme-based enzymes such as lipoxygenase[12] has been observed with exogenous *in vitro* administration of NO. Although human trials of inhaled NO may be justified in clinical situations that, untreated, are likely to be fatal and for which no other therapy is available, attempts at widespread application must await more extensive animal studies of its potential toxicity.

This use of NO as a selective pulmonary vasodilator is only a beginning to the unfathomed clinical applications that may come from our understanding of the role of EDRF/NO in normal physiology and in disease. A brief overview of recent findings will allow an appreciation of the vast clinical implications of this peculiar new cell messenger.

NO may serve multiple functions within the central nervous system.[13–15] The central neurotransmitters acetylcholine, glutamate, and glycine have been known for some time to cause elevation of cyclic GMP levels in the brain. The L-arginine-to-EDRF/NO pathway is now known to be the mechanism by which cyclic GMP is stimulated by these neurotransmitters. This L-arginine-to-NO pathway also has been shown to mediate nonadrenergic, noncholinergic neurotransmission.[16] EDRF/NO also has been implicated in the peripheral analgesic effect of acetylcholine[17] and in peripheral nociceptive mechanisms.[18] In addition to its function as a neurotransmitter, NO, as a free radical, has recently been suggested to be involved in the neurocytotoxicity of Alzheimer's disease, Huntington's disease, and cerebral ischemia.[19]

In contrast to the constitutive form of EDRF/NO synthase discussed above, macrophages contain an inducible form of the enzyme, which appears to be less tightly regulated and which is capable of NO synthesis for extended periods of time.[20] This enzyme is induced in response to endotoxin (lipopolysaccharide) and several cytokines, including γ interferon and tumor necrosis factor. Although this form of the enzyme is present in the macrophage under basal conditions, it is present in endothelium or vascular smooth muscle only following induction by cytokines.[21] This enzyme now appears to be inducible in a wide range of cell types. The NO produced by macrophages appears to perform a "killer" function important to immune defenses.[22,23] The induction of this form of EDRF/NO synthase in endothelium and vascular smooth muscle during endotoxin shock is believed to be related to the severe hypotension observed during sepsis.[24,25] The massive production of NO also may contribute to the cytotoxicity of sepsis and other disease states, including hepatic cirrhosis,[26,27] and has been implicated in ischemia–reperfusion injury and reactive hyperemia.[28,29]

The physiologic and pathophysiologic significance of EDRF has been most extensively investigated in the vas-

cular system, where EDRF activity is present from conduit arteries to microvessels and is conserved across species. The tonic release of EDRF plays an important role in modulating systemic and pulmonary vascular resistance. The EDRF/NO synthase inhibitor, N^G-monomethyl-L-arginine, administered intravenously to rats, rabbits, or guinea pigs results in a marked hypertension that is immediately reversible by an excess of L-arginine.[30] This increased blood pressure is accompanied by a decrease in the vascular conductance of the renal, mesenteric, carotid, and hind quarters vascular beds of conscious, chronically instrumented rats.[31] Most importantly, these changes persisted through 6 h of N^G-monomethyl-L-arginine infusion, demonstrating an inability of vascular regulatory mechanisms to reaccommodate blood flow toward pretreatment levels, as would normally occur with exogenously administered vasoconstrictors. Such a profound response suggests that the basal release of EDRF is a critical component of local vascular control. The production of EDRF in response to increased blood flow has been demonstrated to be the mechanism responsible for the flow-induced vasodilation of coronary arteries and other vessels.[32,33]

EDRF release and activity are markedly impaired in atherosclerosis and hypertension in humans and in a wide variety of animal models,[3,4,34-36] and this messenger molecule also may be involved in the pathogenesis of these disease states. EDRF/NO inhibits the release of renin[37] and prevents proliferation of vascular smooth muscle cells by cyclic GMP–mediated mechanisms.[38] In addition, EDRF/NO inhibits the release of mitogens from human platelets.[39] Impairment of EDRF/NO production has been observed in pregnancy-induced hypertension.[40]

Decreased EDRF activity may play a critical role in the etiology of coronary vasospasm.[3,4,41] Human atherosclerotic coronary arteries have a diminished or obliterated EDRF response. Serotonin, norepinephrine, and other contractile agonists normally cause the release of EDRF from endothelium, which modulates their direct contractile responses.[42] Serotonin, for example, induces a sixfold greater contraction of endothelium-denuded coronary arteries compared to those with an intact endothelium. Aggregating platelets release a wide variety of vasoactive agents, including the endothelium-dependent dilators, serotonin, thrombin, and adenosine diphosphate. In the absence of endothelium, the net effect of aggregating platelets is to vasoconstrict coronary arteries, in contrast to the vasodilation that is observed when the coronary endothelium is intact. Another very important physiologic action of EDRF/NO is the inhibition of both platelet aggregation and adhesion.[3] Thus, impaired endothelial cell function—through extensive intimal damage by atherosclerosis or as a result of ischemia—will promote vasospasm as well as clot formation through increased platelet aggregation and adhesion. Impaired EDRF production has also been demonstrated following balloon angioplasty[43] as well as in internal mammary and venous coronary bypass grafts following their manipulation and preparation for grafting.[44]

The binding of EDRF/NO by hemoglobin has been implicated in the vasospasm following subarachnoid hemorrhage. Endothelium-dependent relaxation of the canine basilar artery has been shown to be inhibited by hemoglobin and by cerebrospinal fluid obtained from patients with subarachnoid hemorrhage.[45]

EDRF/NO is also important in the physiology and pathophysiology of the pulmonary circulation, where it contributes significantly to the low resting tone characteristic of this vascular bed.[46] Endothelial damage and impaired EDRF production have been proposed as contributing factors in both acute and chronic pulmonary hypertension.[46] The modulation of EDRF production by high and low oxygen tensions is especially important in the pulmonary circulation. Hypoxia inhibits EDRF synthase by limiting the availability of molecular oxygen, a substrate for the enzyme, whereas hyperoxia reacts with and inactivates EDRF/NO following its production.[47] Inhibition of EDRF by hypoxia may contribute to hypoxic pulmonary vasoconstriction, and decreased EDRF production due to endothelial damage and hypoxia in areas of pulmonary inflammation may be important in maintaining ventilation–perfusion matching.

Both local[48] and inhalational anesthetics[49,50] have been shown to inhibit EDRF production in isolated blood vessels. Given the wide-ranging functions of EDRF/NO, this action of local and inhalational anesthetics is especially intriguing. Is this a significant component of the vascular actions of anesthetics *in vivo*? Are the effects of anesthetics altered in disease states that impair endothelial cell function, such as hypertension, atherosclerosis, adult respiratory distress syndrome, cerebral vasospasm, or ischemia–reperfusion injury? By inhibiting EDRF/NO production, do anesthetics alter immune function? As the specific functions of EDRF/NO as a neurotransmitter become apparent, it will be especially tantalizing to explore whether inhibition of this L-arginine-to-EDRF pathway plays a role in the central nervous system effects of anesthetics.

EDRF/NO is a newly recognized cellular messenger responsible for the activation of soluble guanylate cyclase and the production of cyclic GMP. It is responsible for the control of vital functions in the vascular, immune, and central nervous systems. Its absence or excessive production have been implicated in a wide variety of disease states. Fratacci *et al.*[8] have clearly demonstrated that NO has direct clinical applications as a selective pulmonary vasodilator. Further clinical applications of NO, of ana-

logues of NO, and of newly developed inhibitors of EDRF/NO synthesis are sure to be forthcoming.

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