Nitric Oxide Donors and Cardiovascular Agents Modulating the Bioactivity of Nitric Oxide
An Overview

Louis J. Ignarro, Claudio Napoli, Joseph Loscalzo

Abstract—Nitric oxide (NO) mediates multiple physiological and pathophysiological processes in the cardiovascular system. Pharmacological compounds that release NO have been useful tools for evaluating the pivotal role of NO in cardiovascular physiology and therapeutics. These agents constitute two broad classes of compounds, those that release NO or one of its redox congeners spontaneously and those that require enzymatic metabolism to generate NO. In addition, several commonly used cardiovascular drugs exert their beneficial action, in part, by modulating the NO pathway. Here, we review these classes of agents, summarizing their fundamental chemistry and pharmacology, and provide an overview of their cardiovascular mechanisms of action. (Circ Res. 2002;90:21-28.)

Key Words: oxidant stress ■ cardiovascular pharmacology ■ endothelium, vascular type ■ nitric oxide

Dysfunction of the normally protective endothelium is found in several cardiovascular diseases, including atherosclerosis, hypertension, heart failure, coronary heart disease, arterial thrombotic disorders, and stroke.1-6 Endothelial dysfunction leads to nitric oxide (NO) deficiency,1,6-11 which has been implicated in the underlying pathobiology of many of these disorders (NO insufficiency states). (For a detailed overview of the role of NO in cardiovascular biology and pathobiology, the reader is referred to Loscalzo and Vita.3) NO insufficiency may reflect an absolute deficit of NO (synthesis), impaired availability of bioactive NO, or enhanced NO inactivation. Whatever its biochemical basis, NO insufficiency limits NO-mediated signal transduction of normal or protective physiological processes. In light of this pathobiology, replacement or augmentation of endogenous NO by exogenously administered NO donors has provided the foundation for a broad field of pharmaco-therapeutics in cardiovascular medicine.

Organic nitrate and nitrite esters represent a time-honored class of NO-donating agents used in cardiovascular therapeutics since the 19th century. These agents have direct vasoactive effects that have been used to treat ischemic heart disease, heart failure, and hypertension for many years. Treatment with conventional nitrate preparations is limited by their therapeutic half-life, systemic absorption with potentially adverse hemodynamic effects, and drug tolerance.1,6 To overcome these limitations, novel NO donors that offer selective effects, a prolonged half-life, and a reduced incidence of drug tolerance have been developed. NO donors are pharmacologically active substances that spontaneously release, or are metabolized to, NO or its redox congeners. In the present article, we review our current understanding of NO-donating compounds and of cardiovascular drugs that modulate the bioactivity of endogenously produced NO.

NO Donors

Because of the limited utility of authentic NO gas in many experimental systems and the short half-life of NO in vivo, compounds that have the capacity to release NO have been widely used as therapeutic agents and as pharmacological tools to investigate the role of NO in cardiovascular physiology and pathophysiology. Several NO donors have been used in clinical settings for decades (e.g., nitroglycerin and nitrates). However, the growth of interest in the physiology of NO since the mid-1980s has led to the development of a variety of new NO donors that offer several advantages over conventional NO donors.

The pathways leading to enzymatic and/or nonenzymatic formation of NO differ greatly among individual compound classes, as do their chemical reactivities and kinetics of NO release. Moreover, because the reaction of NO with oxygen is a third-order kinetic process, the oxidation of NO to nitrate and nitrite is exquisitely sensitive to the local oxygen tension, as is the extent of derivative side reactions, such as nitrination and/or nitrosation of biomolecules. This basic biochemistry is further complicated by compound-specific formation of intermediate and end products that may arise during metabolism and/or degradation, at times in amounts far exceeding...
those of NO.\textsuperscript{1,7,8} In living systems, however, the redox form of nitrogen monoxide that is actually released (namely, nitroxy anion [NO\textsuperscript{−}], NO radical [NO], or nitrosonium [NO\textsuperscript{+}]) primarily defines the reactivity of the NO donor toward other biomolecules, the profile of byproducts, and the bioactivity of the donor.\textsuperscript{9} These simple chemical principles are likely to account for much of the discrepancy in experimental results obtained by using the same cell or tissue preparation but different NO donors and, in part, for the variability in clinical responses to different nitrovasodilators.

Direct Donors
Direct NO donors are pharmacological agents with either a nitroso or nitrosyl functional group. In contrast to organic nitrates, which require metabolism for activity,\textsuperscript{10} these agents spontaneously release NO\textsubscript{X} (defined as the range of redox forms of nitrogen monoxides generated by the donor molecule). Three common members of this class of agents are NO gas, sodium nitroprusside, and sodium trioxodinitrate (Angeli’s salt). As the most direct form of the endogenous molecule, NO gas is freely soluble in physiological solutions. Because of its short half-life and rapid reactivity toward molecular oxygen, its use has been limited to inhalation therapy for pulmonary vascular disorders, for which it has met with some limited success.\textsuperscript{11}

In sodium nitroprusside, NO is coordinated as a nitrosyl group liganded to iron in a square bipyramidal complex\textsuperscript{1} and is released spontaneously at physiological pH from the parent compound. Sodium nitroprusside has been used effectively for decades for the treatment of hypertension and heart failure. The use of this nitrovasodilator is limited by the need to administer it parenterally, by tolerance, and by the potential for the development of thiocyanate toxicity with prolonged administration (in rhodanase-deficient individuals).

Angeli’s salt and p-nitrosophosphate compounds (−NO heterodienophiles) are agents that generate NO\textsuperscript{−} spontaneously under physiological conditions.\textsuperscript{12} This reduced form of NO has unique effects on vascular smooth muscle distinct from other redox forms of NO.\textsuperscript{13} NO\textsuperscript{−} donors have not yet been tested in human subjects.

Diethylenetriamine/NO and diethylenetriamine/NO are compounds of the diazeniumdiolate or NONOate [N(O)NO] class, in which NO is covalently linked to diethylenetriamine and, respectively, and spontaneously released as NO\textsuperscript{−}.\textsuperscript{14} Diazenuimdiolates have been shown to provide neuroprotection from hydrogen peroxide–induced cortical neurotoxicity,\textsuperscript{15} to reverse cerebral vasospasm,\textsuperscript{16} and to reduce pulmonary vascular pressures and improve oxygenation in acute lung injury\textsuperscript{17}; this latter benefit was observed using an aeropolized preparation. Other heterocyclic NO-releasing compounds include those of the oxatriazolium class (sydnonimines) and the furoxan class.\textsuperscript{18} These two classes of NO donors require cofactors to facilitate NO release: the sydnonimines require oxidants, such as molecular oxygen, and the furoxans require thiols. 3-Morpholinosydnonimine, derived from molsidomine, is a zwitterionic compound formed by combining morpholine and a sydnonimine\textsuperscript{19} that spontaneously decomposes at physiological pH into NO\textsuperscript{−} and superoxide anion (see Figure 1).

Newer NO donor drugs, in particular the S-nitrosothiols, offer advantages over the existing drugs because they do not share the drawbacks of organic nitrates and nitroprusside, including a limited capacity for inducing oxidant stress or tolerance in vascular cells.\textsuperscript{20} Initial small clinical studies suggest that they may be of benefit in a variety of cardiovascular disorders.\textsuperscript{21} S-Nitrosothiols are a class of NO-donating compounds that are naturally occurring and spontaneously release NO\textsuperscript{−}; they may also gain access to the intracellular compartment by the catalytic action of plasma membrane–bound protein disulfide isomerase and associated transnitrosation reactions.\textsuperscript{22} Members of this class of agents include S-nitroso-glutathione, S-nitroso-N-acetylpenicillamine, and S-nitroso-albumin\textsuperscript{23} (see Figure 2).

NO is also generated in vivo nonenzymatically under a variety of (patho)biological conditions. L-Arginine (and d-arginine) yields NO after reaction with peroxides.\textsuperscript{1,7,8} In addition, under acidic (ischemic) conditions or in the presence of sufficient reducing equivalents, nitrite can be reduced directly to NO.\textsuperscript{24}

Donors Requiring Metabolism
The classic nitrovasodilators, organic nitrate and nitrite esters, including nitroglycerin, amyl nitrite, isosorbide dinitrate, isosorbide 5-mononitrate, and nicorandil (Figure 3), have been used for many years in the treatment of cardiovascular diseases.\textsuperscript{25–28} Their principal action is vasorelaxation, mediated by guanylyl cyclase activation and by direct inhibition of nonspecific cation channels in vascular smooth muscle cells (VSMCs). As such, these agents represent the prototypical form of NO-replacement therapy. The limitations of this class of agents are well known and include potentially adverse hemodynamic effects, drug tolerance, lack of selectivity, and limited bioavailability. Notwithstanding these shortcomings, prudent use of these agents yet represents the mainstay of therapy for patients with ischemic heart disease. The first
reports of the clinical use of organic nitrates and nitrites was derived from the work of Brunton 28a in 1867 and the seminal work of Murrell 29 in 1879, which showed the clear benefits of nitroglycerin in the treatment of angina pectoris. All of the organic nitrate esters used since Murrell’s demonstration of the benefits of nitroglycerin are prodrugs requiring enzymatic metabolism to generate bioactive NO. The major enzyme system involved is located within microsomal membranes, has an estimated apparent molecular mass of 160 kDa, and manifests enhanced activity in the presence of reducing equivalents, especially thiols.30 Although the enzyme has not been more specifically characterized, growing evidence suggests that the cytochrome P-450 system, in conjunction with NADPH and glutathione-S-transferase activities, is required for the linked metabolic processes of denitration and reduction of organic nitrate esters to authentic NO.31,32 Importantly, thiols potentiate the action of organic nitrate esters.33,34 Tolerance limits the clinical use of organic nitrite and nitrate esters; it is associated with increased angiotensin II–dependent vascular production of superoxide anion from NAD(P)H oxidase and endothelial NO synthase (eNOS).35,36 The superoxide anion generated by these enzymes reacts with NO derived from the NO donor to form peroxynitrite (OONO·), as indicated by the finding of increased urinary 3-nitrotyrosine in nitrate-tolerant patients.37 Importantly, nitrate tolerance is also associated with cross-tolerance to endothelium-derived NO,38 both by the oxidative inactivation of this endogenous NO to peroxynitrite and by the “uncoupling” of eNOS activity.39 Low-molecular-weight thiols, ascorbate, L-arginine, tetrahydrobiopterin, hydralazine, ACE inhibitors, and folate have been successfully used to reverse or prevent nitrate tolerance.40 Mutagenic effects of NO donors have also been demonstrated. In a study by Birnboim and Privora,41 glyceryl trinitrate and sodium nitroprusside appeared to promote mutagenesis in a glutathione-dependent manner, as shown with the use of a very sensitive detection system. N-Acetylcysteine and oxothiazolidine-4-carboxylate reduced the mutagenicity of the NO donors.

The mechanisms of metabolism and action of the principal classes of NO donors are illustrated in Figure 4.

Bifunctional Donors
Recognizing that nitrate esters and S-nitrosothiols represent the essential functionality of many NO donors, investigators have chosen to modify existing pharmacological agents with these functional groups in an effort to exploit some of the beneficial effects of NO without limiting the pharmacological effect of the parent compound. Early work with S-nitroso-
Nitroaspirins

Nitroaspirins are nitrate ester compounds and include 2-acetoxybenzoate 2-(2-nitroxy-methyl)-phenyl ester (NCX-4016) and 2-acetoxybenzoate 2-(2-nitroxy)-butyl ester (NCX-4215) (Figure 5). NCX-4016 is a stable compound that requires enzymatic hydrolysis to liberate NO, and the kinetics of this metabolic processing leads to durable production of NO released at a constant rate from the site of metabolism.50 This agent is orally bioavailable as a prodrug, producing significant levels of diclofenac in plasma with a half-life of 51 hours have been applied to vascular grafts66 to prevent thrombosis and restenosis. Similarly, the N(O)NO group has been incorporated into polymeric matrices synthesized to modulate the time course of NO release67; this approach showed potent antiplatelet activity in a vascular graft in baboons.67

BSA can be modified covalently to bear multiple S-NO groups, which possess vasodilatory and antiplatelet properties.23,68 Poly-S-nitrosated BSA applied locally to a site of vascular injury reduced restenosis in a rabbit model.69 Local delivery of poly-S-nitrosated BSA, compared with BSA alone, induced a 50% to 70% reduction in platelet attachment and surface activation, together with a 40% reduction in

S-Nitroso-NSAIDs

S-Nitroso-diclofenac (Figure 2), as a prototype of the S-nitroso ester class of NSAIDs that release NO (probably as NO−), has recently been shown to possess unique properties.53 This agent is orally bioavailable as a produg, producing significant levels of diclofenac in plasma with a half-life of 51 hours after oral administration to mice. In addition, S-nitroso-diclofenac has equipotent anti-inflammatory and analgesic properties as diclofenac but is gastric-sparing compared with the parent NSAID. Thus, S-nitrosothiol esters of diclofenac and other NSAIDs constitute a novel class of NO−-donating compounds with uncompromised anti-inflammatory and analgesic properties but a markedly enhanced gastric safety profile. The use of NO-NSAIDs for general analgesic and anti-inflammatory purposes or for primary or secondary cardiovascular prevention awaits the results of ongoing clinical trials.

Delivery Systems and Drug Targeting

There has been considerable interest in developing NO delivery systems that can be used to target drug action and modulate the kinetics of drug release. Drug-eluting vascular stents with a variety of coatings (including fibrin, heparin, and multiple polymers) that contain NO donors have been tested with variable effects.64,65 NO-containing cross-linked polyethyleneimine microspheres that release 10 nmol NO/mg with a half-life of 51 hours have been applied to vascular grafts66 to prevent thrombosis and restenosis. Similarly, the N(O)NO group has been incorporated into polymeric matrices synthesized to modulate the time course of NO release67; this approach showed potent antiplatelet activity in a vascular graft in baboons.67

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The major limitation for the long-term use of NSAIDs is their ability to cause gastrointestinal toxicity. Indeed, even low-dose aspirin maintains its ability to damage the gastric mucosa by inducing erosions and bleeding,44,45 and this effect appears to be closely related to the inhibition of cyclooxygenase (I). Prostaglandins and NO are thought to play a major role in maintaining mucosal integrity. NO has cytoprotective properties46,47 in the stomach and other organs that are derived from its ability to increase local blood flow and to scavenge destructive free radicals.48,49 New classes of NSAIDs that release NO, called NO-NSAIDs, have been developed and have been shown to be safe and effective alternatives to conventional NSAIDs.50,51 These agents are composed of two classes of compounds, one that contains a nitrate ester functionality50–52 and one that contains an S-nitrosothiol functionality.53
neointimal area. The advantages of the use of this agent include the avidity of the subendothelial matrix for albumin (ie, targeting), its long half-life in vivo, and its ability to serve as a local depot of NO, requiring trans-3-nitrosation reactions (thiol-S-nitrosothiol exchange) to deliver NO via low-molecular-weight S-nitrosothiol intermediates.

### Cardiovascular Agents Modulating Endogenous NO Bioactivity

#### ACE Inhibitors
Angiotensin II and bradykinin levels within the vascular wall are controlled by ACE. ACE degrades bradykinin and generates angiotensin II; in turn, bradykinin stimulates the endothelium to release vasodilating substances, in particular, NO. Thus, by potentiating bradykinin, ACE inhibitors may promote the release of endothelial NO. Indeed, ACE inhibitors have been shown to exert some of their beneficial pharmacological effects by increasing vascular NO activity. In addition, because of the significant constitutive expression of NO synthase in the juxtaglomerular apparatus, NO appears to act as a tonic enhancer of renin secretion via cGMP-dependent inhibition of Rho. Moreover, angiotensin II can stimulate superoxide production, which reduces the bioavailability of NO; an event that can be blocked by ACE inhibitors.

ACE inhibitors improve endothelial function in the subcutaneous, epicardial, and renal circulation but are ineffective at potentiating the blunted response to acetylcholine in the forearm of patients with essential hypertension (see review). This effect may also revert to an inhibitory effect compatible with the inhibition of renin secretion by cGMP-dependent protein kinase(s). Moreover, angiotensin II can stimulate superoxide production, which reduces the bioavailability of NO; an event that can be blocked by ACE inhibitors.

#### Calcium Channel Blockers
Dihydropyridine calcium channel antagonists have been used for many years in the treatment of angina pectoris and hypertension. Their mechanism of action is based on inhibition of the smooth muscle L-type calcium current, thereby decreasing intracellular calcium concentration and inducing smooth muscular relaxation. Dihydropyridines can also induce the release of NO from the vascular endothelium. The dihydropyridine calcium antagonists can reverse impaired endothelium-dependent vasodilation in different vascular beds, including the subcutaneous, epicardial, and peripheral arteries of the forearm circulation. In the forearm circulation, nifedipine and lacidipine can improve endothelial dysfunction by restoring NO availability. In addition, in several experimental preparations, including microvascular and macrovascular studies, the sensitivity of the vasorelaxing effect of the dihydropyridines to inhibitors of NO synthase, such as N\(^\text{G}\)-nitro-l-arginine or N\(^\text{G}\)-nitro-l-arginine methyl ester, has been clearly demonstrated. These studies show that the NO-releasing effect is not unique to nitrendipine but is a class phenomenon shared by the dihydropyridines and several nondihydropyridine calcium channel antagonists. The underlying mechanism of NO release evoked by these drugs is not entirely clear but may involve modulating endothelial membrane potential via a myoendothelial interaction, up-regulating eNOS expression, increasing the activity of endothelial superoxide dismutase(s), and enhancing the flow-mediated release of endothelial NO via vascular smooth muscle relaxation and vasodilation. These findings of a dual mode of action, ie, the direct relaxing effect of inhibiting smooth muscle L-type calcium channels and the indirect relaxing effect of releasing NO from vascular endothelium, may help explain the beneficial antihypertensive effect of the dihydropyridine calcium channel antagonists.

#### Statins
The efficacy of the widely prescribed 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) in decreasing the incidence of cardiac events and mortality is likely enhanced by their possible antioxidant properties and their ability to upregulate eNOS expression and activity. Statins also reverse the downregulation of eNOS expression induced by hypoxia and oxidized LDL which may underlie their capacity to improve the vascular bioactivity of NO and plaque stability independent of their lipid-lowering effects. By inhibiting L-mevalonate synthesis, statins also reduce the synthesis of farnesylpyrophosphate and geranylgeranylated phosphos (GGPP). GGPP is important in the posttranslational modification of a variety of proteins, including eNOS and Ras-like proteins, such as Rho. Inhibition of Rho results in a 3-fold increase in eNOS expression and nitrite generation. The effect of statins on eNOS expression is reversed by GGPP but not by farnesylpyrophosphate or LDL cholesterol. Thus, an important non–cholesterol-lowering effect of statins is the upregulation of eNOS expression via the inhibition of Rho. Moreover, statins prevent the downregulation of eNOS induced by tumor necrosis factor-α. These effects may play an important role in the setting of chronic statin therapy for the primary and secondary prevention of coronary heart disease.

Simvastatin was recently found to attenuate brain injury and cerebral infarct size in mice and to limit cardiac dysfunction after ischemia/reperfusion injury. In these latter studies, the protective effects of simvastatin were related to the increased efficiency of the NO pathway. Thus, the statins can now be considered as agents that both modulate the synthesis and enhance the bioactivity of endogenous NO. Simvastatin can also activate the protein kinase Akt to promote new blood vessel growth, which may serve as an additional beneficial mechanism in individuals with atherosclerotic disease.

#### β-Blockers
β-Blockers may also interfere with the NO pathway. For example, nebivolol, a β1-blocker and a chemical racemate...
that contains equal proportions of D- and L-enantiomers, was found to induce endothelium-dependent arterial relaxation in dogs in a dose-dependent fashion. The endothelium-dependent relaxation induced by nebivolol is abolished by N\textsuperscript{G}-nitro-L-arginine methyl ester, an inhibitor of NO synthase. These experimental studies were further supported by studies involving infusion of nebivolol into the phenylephrine-precontracted superficial hand veins of healthy volunteers, yielding dose-dependent venodilation. Similarly, nebivolol increased forearm blood flow, measured by venous occlusion plethysmography, by \( \approx 90\% \). Whether this compound or related compounds can enhance NO production in essential hypertensives or in other individuals with cardiovascular diseases remains undetermined at this time.

**Phosphodiesterase Inhibitors**

Sildenafil is a selective inhibitor of phosphodiesterase type 5 that is orally effective in the treatment of erectile dysfunction. Its pharmacological actions are a consequence of prolonging the signaling actions of NO because this drug prevents cGMP hydrolysis by inhibition of a cGMP phosphodiesterase V subtype enriched in penile smooth muscle. Coadministration of sildenafil with isosorbide mononitrate or nitroglycerin produces significantly greater reductions in blood pressure than nitrates alone provide in patients with stable angina. On the basis of these data, sildenafil should not be administered to patients taking nitrates because there is a small, but finite, increased risk of developing ischemia or infarction with sexual activity. Thus, before prescribing sildenafil for erectile dysfunction in patients with known cardiac disease or multiple cardiovascular risk factors, physicians should discuss the potential cardiac risk of sexual activity and perform an appropriate medical assessment, including an exercise stress test if appropriate.

To date, the actions of sildenafil in vascular disorders distinct from that of erectile dysfunction have yet to be studied adequately. An alternative NO-based approach for erectile dysfunction therapy has recently been indicated from evidence that pathways inhibiting erection and favoring smooth muscle contraction are mediated by adrenergic nerves. Nitrosoated \( \alpha \)-adrenergic receptor antagonists have been developed that contain an \( \beta \)-nitrosothiol functionality linked to an \( \alpha \)-adrenergic receptor antagonist (yohimbine and moxisylyte) by an inert organic-ester tether. The rationale behind the development of this agent is that the NO donor prompts early (immediate) vasodilation while the \( \alpha \)-adrenergic blocker maintains the vasodilator effect. Pharmacological demonstration of these NO donor properties in relaxing human penile smooth muscle, their \( \alpha \)-adrenergic antagonism, and their ability to induce erection in laboratory animals suggest that NO-releasing adrenergic receptor antagonists may be useful as bifunctional agents for local treatment of erectile dysfunction.

A summary of the effects of cardiovascular agents that modulate endogenous NO bioactivity is provided in Figure 6.

**Concluding Remarks**

Exogenous NO-donating agents clearly can elicit beneficial actions relevant to cardiovascular disorders. Although more than 20 years has passed since the identification of NO as an endogenous substance produced by the cardiovascular system, attempts toward developing accepted therapeutic approaches for modulating endogenous NO production or activity have not progressed much beyond the early work of the last century until recently. Understanding the complex chemistry, biochemistry, and molecular biology of NO and its signaling responses, developing targeted therapies for delivery of NO or agents that enhance endogenous NO production, and choosing the optimal adjunctive therapies that potentiate the benefits of NO donors or endogenous NO are all issues that require additional clinical study. Further thoughtful, rational developments in this fertile area of cardiovascular investigation hold promise for enhancing the therapeutic armamentarium for a variety of cardiovascular disorders.

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