Nitric Oxide and Nitrovasodilators: Similarities, Differences and Potential Interactions

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Many similarities exist between the exogenous nitrates and endothelium-derived relaxing factor, which is nitric oxide or a thiol derivative. Both act by way of guanylate cyclase, which increases intracellular concentrations of cyclic guanosine monophosphate, resulting in smooth muscle cell relaxation and antiplatelet effects. ThiolS may be important in the biotransformation of exogenous nitrates and other intracellular processes involving nitric oxide. As such, important interactions might be expected between nitrates and endothelium-dependent processes that involve nitric oxide. This review explores the mechanisms of action, biologic effects and potential interactions between nitrates and endothelium-derived relaxing factor.

Chemical Nature of Endothelium-Derived Relaxing Factor

In 1980, Furchgott and Zawadzki (2) discovered that the acetylcholine-induced relaxation of rabbit aortic strips required an intact endothelium. They concluded that acetylcholine stimulated muscarinic receptors on the endothelium, leading to the release of a nonprostanoid relaxant factor, which they named endothelium-derived relaxing factor. A diffusible substance with a half-life of several seconds, (6,7) endothelium-derived relaxing factor could be inhibited by hemec containing proteins (8), and it was destroyed by oxygen free radicals (10). It also activated soluble guanylate cyclase and increased the concentration of cyclic guanosine monophosphate (cyclic GMP) (10). Collectively, these characteristics led to the simultaneous observation by Ignarro et al. (4) and Furchgott and Khan (5) that endothelium-derived relaxing factor was strikingly similar to nitric oxide.

The first proof that nitric oxide was at least one of the relaxing factors resulted from studies by Palmer et al. (11). Using a chemiluminescence assay, they demonstrated that cultured endothelial cells release nitric oxide when exposed to bradykinin. In this assay, nitric oxide is specifically detected by its reaction with ozone. Results of these studies provided strong evidence that endothelium-derived relaxing factor is nitric oxide or a chemically related compound. Recent findings suggest that endothelium-derived relaxing factor may also be similar to a labile nitroso compound (S-nitroso-L-cysteine) (3,12).

Mechanism of Action of Endothelium-Derived Relaxing Factor and Organic Nitrates

Endothelium-derived relaxing factor. The mechanism of action of nitric oxide has been examined in considerable detail (10,13). Palmer et al. (14) have demonstrated that nitric oxide is synthesized from the guanidino nitrogen of the amino acid L-arginine. This conversion is controlled by an enzyme, nitric oxide synthase, which can be stimulated by a
number of physical and chemical stimuli and is inhibited by several arginine analogs, including N\textsuperscript{G}-monomethyl-L-arginine. Receptors on the endothelial cell membrane modulate this interaction in association with signal transduction proteins (the G protein family) (15). Serotonin receptors, for example, interact with the pertussis-sensitive inhibitory protein (GI) to modulate release of nitric oxide (16). After release from the endothelium, endothelium-derived relaxing factor diffuses across the extracellular space into the smooth muscle cell and utilizes the guanylate cyclase second-messenger system to implement its biologic actions. Both nitric oxide and endothelium-derived relaxing factor have been shown to activate soluble guanylate cyclase, leading to an increase in the intracellular concentration of cyclic GMP (10,17).

Cyclic guanosine 3',5'-monophosphate system: intracellular second messenger. Cyclic GMP is formed from guanosine 5'-triphosphate by the activation of guanylate cyclase, of which there are at least three distinct types: 1) soluble guanylate cyclase, which is activated by nitric oxide and organic nitrates; 2) membrane-bound (particulate) guanylate cyclase, which is induced by atrial natriuretic peptide; and 3) cytoskeleton guanylate cyclase, which is sensitive to E. coli heat-stable enterotoxin (18). Purified soluble guanylate cyclase has been shown to contain heme, which is now thought to be the receptor site for nitric oxide on soluble guanylate cyclase (19–21). Electron paramagnetic resonance studies have demonstrated that the binding of nitric oxide to heme results in a structural change of the heme molecule in which the iron atom protrudes out of the plane of the protoporphyrin ring (22). Local thiol groups are necessary to reduce the heme iron atom to the ferrous state, thus facilitating the nitric oxide–heme interaction.

The net result of the activation of soluble guanylate cyclase is the formation of cyclic GMP, which then activates cG-Pk, resulting in smooth muscle relaxation. Cyclic GMP also stimulates calcium influx and uptake by intracellular stores. It may also inhibit calcium influx across the plasma membrane by decreasing production of diacyl glycerol (DAG) and inositol 1,4,5-triphosphate (IP\textsubscript{3}), which results in a decrease in protein kinase C activity (Fig. 1) (24). The effects of cyclic GMP are terminated by a specific phosphodiesterase (25).

Several inhibitors of this pathway have been utilized in studies examining the mechanism of action of endothelium-derived relaxing factor. Because of the natural affinity of nitric oxide for heme rings, free reduced hemoglobin has been used as an inhibitor of both endothelium-derived relaxing factor (8,26) and nitric oxide (27). Methylene blue has also been used to inhibit endothelium-derived relaxing factor by the direct inhibition of guanylate cyclase or by the generation of superoxide free radicals (28,29).

Nitrovasodilators. The nitrate compounds used in the treatment of angina have been termed organic nitrovasodilators because they possess a nitrate ester bond (R–O–nitric oxide\textsubscript{2}) (Fig. 2). This distinguishes them from nitric oxide-containing compounds without an ester bond, such as nitroprusside. Important similarities and differences exist in the biotransformation of these organic and inorganic nitrates, but it is generally accepted that they share a final common pathway with endothelium-derived relaxing factor.

The mechanism by which nitroprusside releases nitric oxide has recently been reported. It has been previously thought that nitric oxide release occurred spontaneously (30). However, more recently Bates et al. (31) have reported that a one-electron reduction with accompanying cyanide loss was required before nitric oxide could be released.

The biotransformation of organic nitrates is probably more complex. Although there is much controversy about the role of thiols, they may be important at several levels in the nitrate/nitric oxide pathway including 1) the formation of S-nitrosothiol intermediaries, and 2) the activation of soluble guanylate cyclase by nitric oxide. The formation of nitrosothiol intermediaries from organic nitrates seems to depend on the deamination of the R–O–nitric oxide\textsubscript{2} to a reactive thiol intermediate (32,33). Using a rat model of tolerance, Fung et al. (34) showed that the beneficial effect of the thiol donor N-acetylcysteine on nitroglycerin might be mediated by extracellular S-nitrosothiol formation in addition to intracellular thiol repletion. A recent study in rats, which used various thiol donors and depleting agents, demonstrated that both intracellular and extracellular thiols are important in modulating the effect of nitroglycerin in vivo (35). Although suggestive, it has not been shown conclusively that nitrothiols are intermediaries in the biotransformation process.

Recent data by Chung and Fung (36) have demonstrated that nitrates are enzymatically converted to yield nitric oxide by a surface membrane enzyme system that is not identical to that provided by glutathione-S-transferases (37). It has been suggested by these investigators and others (38) that sulphydryl-donating compounds may act instead as cofactors in the enzymatic transformation of organic nitrates.

Thiols have also been shown to be potentially important for the antiplatelet effect of the nitrovasodilators. Loscalzo (39) demonstrated that N-acetylcysteine potentiated the platelet inhibitory actions of nitroglycerin by inducing the formation of S-nitroso-N-acetylcysteine. A recent study has reported that the liberation of nitric oxide from isosorbide dinitrate (ISDN) and nitroglycerin is potentiated by cysteine (40). In contrast, the release of nitric oxide from nitroprusside was reported to be independent of cysteine (41). However, thiols can also potentiate the antiplatelet effects of nitroprusside, which suggests that they have an important role at other levels (42). The fact that added thiols potentiate antiplatelet effects does not prove that an important relation exists between thiols and nitrates in vivo in the absence of administered thiols.

The binding of nitric oxide to the heme moiety of soluble guanylate cyclase and the activation of cyclic GMP follows the same pathway as that described for endothelium-derived relaxing factor (10). A recent study in pigs demonstrated
Figure 1. A, Proposed mechanisms by which nitroso compounds are bioconverted to nitric oxide, which binds to the heme moiety of guanylate cyclase (GC), leading to increases in intracellular levels of cyclic guanosine monophosphate (cGMP). Reprinted, with permission, from Stamler and Loscalzo (127). B, Schemata showing some of the potential sites of action of cyclic guanosine monophosphate-mediating vascular smooth muscle cell relaxation. Reprinted, with permission, from Ahlner et al. (112). See text for details. ATPase = adenosine triphosphatase; DAG = diacyl glycerol; GTP = guanosine triphosphate; HONO = nitrous acid; IP3 = inositol 1,4,5-triphosphate; MHC = myosin heavy chain; ML = myosin light chain; MLCK = myosin light chain kinase with active enzyme; MLCKi = MLCK with inactive enzyme; NO = nitric oxide; NO2 = nitrite; NTG = nitroglycerin; PIP2 = phosphatidyl inositol biphosphate; Pk-C = protein kinase C; R-ONO2 = organic nitrate; RSH = reduced thiol; RSNO = S-nitrosothiol; RSSR = disulfide.

inhibition of the antiplatelet effect of nitroglycerin by methylene blue, along with a documented decrease in cyclic GMP level (43). A similar study in humans also demonstrated that nitroglycerin was capable of increasing platelet cyclic GMP levels and that the degree of vasodilation correlated with these levels (44). These studies support the view that the guanylate cyclase system is the final pathway for the actions of all nitrovasodilators.

Differences in tolerance between organic nitrates and nitroprusside. A detailed discussion of tolerance is beyond the scope of this review. Continuous administration of organic nitrates leads to tolerance to their hemodynamic and clinical effects. Needleman et al. (45,46) demonstrated that arterial strips require the presence of tissue thiol groups to relax to nitroglycerin and nitroprusside. Numerous other studies have demonstrated that thiols group donors, such as cysteine and glutathione, can potentiate guanylate cyclase activation by both nitrates and nitroprusside (41,47-49). Moreover,
studies have demonstrated that the administration of thiol donors, such as N-acetylcysteine, or thiol-containing angiotensin-converting enzyme inhibitors, such as captopril, can result in the reversal of nitrate tolerance or the potentiation of nitrate effects in smooth muscle cell preparations (50,51), intact animals (33,52) and clinical studies (53–56). The sulfhydryl depletion hypothesis, which has indirect support from the previous data, is now thought to be incomplete, however. Vascular sulfhydryl groups are not decreased during development of nitroglycerin tolerance (57). The recent data by Fung (58) that show that nitrates are enzymatically converted in smooth muscle cells to nitric oxide have lead to the evolution of the thiol-depletion hypothesis. It may be that tolerance is due in part to a decrease in available sulfhydryl groups to interact with enzymes that are necessary for metabolic activation of nitrates.

It is also clear that long-term nitrate administration is accompanied by a variety of compensatory physiologic effects, including increased plasma concentrations of renin and catecholamines, increased body water and shifts in vascular volume (59–61). It is likely that tolerance is a complex process involving both cellular and physiologic processes.

Cellular tolerance has not been shown to be a major problem for nitroprusside. Nitric oxide is released from nitroprusside by one-electron transfer and may interact with different pools of thiols than those required for the transformation of organic nitrates, but this has not been definitively proved (40).

**Biologic Actions of Endothelium-Derived Relaxing Factor**

**Conduit vessels.** The endothelium modulates vascular tone by releasing various relaxing factors (i.e., endothelium-derived relaxing factor, endothelium-derived hyperpolarizing factor and prostacyclin) and the contracting factors (i.e., thromboxane-A₂, free radicals, endothelium-derived contracting factors and endothelin) (62,63). Endothelium-derived relaxing factor can be released in response to a variety of stimuli, including shear stress and pressure, aggregating platelets, thrombin, adenosine diphosphate (ADP), serotonin, bradykinin, histamine, norepinephrine, vasopressin, substance P and acetylcholine (64).

**Experimental evidence.** A substantial body of basic research has established the importance of endothelium-derived relaxing factor in both basal and stimulated control of vascular tone in large conduit vessels. Studies have demonstrated that basal cyclic GMP levels are higher in aortic strips when the endothelium is intact than when it has been denuded (8), and bioassay techniques have also demonstrated the basal release of nitric oxide from large conduit vessels. The role of endothelium-derived relaxing factor as the mediator of vasodilation is supported by experiments that used endothelium-dependent agonists in the presence of inhibitors of endothelium-derived relaxing factor (8).

Increasing flow is also an important stimulus of endothelium-dependent vasodilation. It has been shown in the canine femoral and coronary artery that removal of the endothelium by balloon injury prevents the flow-mediated increase in diameter, demonstrating that the response is endothelium dependent (65–68).

**Human studies.** In vitro studies using human coronary arteries have shown endothelium-dependent relaxation in response to acetylcholine in normal arteries and impaired relaxation in atherosclerotic coronary arteries (69–71). In vivo studies of conduit vessel vasomotion have focused on the brachial, femoral and coronary circulation (72–74). Ludmer et al. (74) demonstrated coronary vasodilation in response to acetylcholine in patients with normal coronary arteries and no risk factors for atherosclerosis, but vasoconstriction occurred in patients with atherosclerosis (Fig. 3). This was the first study to recognize that endothelium-dependent vasodilation does exist in vivo in normal human subjects and is impaired in patients with atherosclerosis. A number of subsequent studies also showed that other agonists, including substance P, serotonin and histamine, can increase the coronary artery diameter in normal subjects (75–77). Studies using inhibitors of nitric oxide and nitric

![Figure 3. Recent change in vessel diameter with acetylcholine plotted in (A) atherosclerotic and (B) normal coronary arteries.](image-url)
nitric oxide synthase have confirmed that nitric oxide mediates acetylcholine-induced coronary artery dilation (69,78,79).

Flow-mediated vasodilation has also been demonstrated in human femoral, brachial and coronary arteries (72,80,81). Physiologic stimuli that lead to an increase in coronary blood flow, including the use of papaverine or adenosine (82,83), bicycle exercise testing (84), cold pressor testing (85,86) and mental arithmetic (87), have all been shown to increase coronary artery diameter in patients without atherosclerosis or risk factors. An impaired response is seen in patients with atherosclerosis. The parallel response of coronary arteries to the physiologic stimuli and to acetylcholine led investigators to the conclusion that these stimuli were endothelium dependent. However, studies using nitric oxide inhibitors have not been done to confirm the role of nitric oxide in flow-mediated vasodilation in the coronary circulation.

Resistance vessels. Experimental studies. In vitro studies that used microscopy have demonstrated dilation of resistance vessels in response to acetylcholine from rabbit ear (88), rat cremaster muscle (89) and canine coronary resistance vessels (90). In vivo studies using rabbit hind limbs or isolated buffer-perfused rabbit heart and primate studies using nitric oxide inhibitors have confirmed that nitric oxide mediates vasodilation in the microcirculation (91-94).

Studies that used systemic doses of N\textsuperscript{G}-monomethyl-L-arginine have shown an increase in blood pressure and reflex bradycardia in the rabbit (93), rat (96) and guinea pig (97), suggesting that the basal release of endothelium-derived relaxing factor is important at the resistance vessel level in regulating systemic blood pressure.

Human studies. Vasodilation in resistance vessels in vivo is assessed by measuring blood flow. For a constant pressure, "tone" in the resistance vessels is the major determinant of flow. Importantly, any stimulus that relaxes resistance vessels and increases flow will result in a mild increase in conduit vessel diameter as a consequence of flow-mediated vasodilation. Resistance vessel dilation therefore effects changes in conduit vessels through flow-mediated dilation.

Cholinergic agonists infused into the brachial artery are known to increase flow as measured by impedance plethysmography. Vallance et al. (98) demonstrated that this response could be attenuated by the infusion of N\textsuperscript{G}-monomethyl-L-arginine, which suggests that the dilator action of endothelium-derived relaxing factor contributes to the stimulated regional blood flow response to acetylcholine. In the coronary circulation, the blood flow response to acetylcholine and substance P is impaired in the microvasculature of patients with nonobstructive coronary artery disease (99,100). Lefroy et al. (79) used coronary sinus oxygen saturation as a measure of coronary blood flow to demonstrate that N\textsuperscript{G}-monomethyl-L-arginine did not abolish the blood flow increase in response to acetylcholine. They suggested that endothelium-derived relaxing factor may not be the only mediator of acetylcholine-induced increase in blood flow in the coronary microvasculature. Other mediators, such as endothelium-derived hyperpolarizing factor, may be involved, or the contribution of nitric oxide is small and was not detected because of the inherent variance in a biologic system.

Basal effects of endothelium-derived relaxing factor on the microvasculature in humans have been addressed by studies using endothelium-derived relaxing factor inhibitors. Vallance et al. (98) demonstrated a 50% decrease in forearm blood flow to locally infused N\textsuperscript{G}-monomethyl-L-arginine, which suggests a role for endothelium-derived relaxing factor in the maintenance of resistance vessel tone. Lefroy et al. (79) showed that N\textsuperscript{G}-monomethyl-L-arginine produced only a small, nonsignificant decrease in basal flow. Thus, it is generally believed that endothelium-derived relaxing factor plays a role in the stimulated and basal maintenance of tone in the resistance vessels, but the story is far from complete.

Platelet function. The antiplatelet effects of nitric oxide and endothelium-derived relaxing factor have been known for some time (42,101). The role of endothelium-derived relaxing factor as an antiplatelet agent was shown by Radiomski et al. (102) and may be an important action of endothelium-derived relaxing factor because prostacyclin has potent antiaggregatory properties but only mildly antiplatelet function. There is additional evidence that endothelium-derived relaxing factor can disaggregate platelets (42), and prostacyclin has been shown to potentiate this antiaggregatory and disaggregating activity (102,103). Endothelium-derived relaxing factor is also known to increase cyclic guanosine monophosphate level in platelets, but the mechanism by which this inhibits platelet aggregation and adhesion is poorly understood. Studies have suggested that a reduction in calcium influx may inhibit the phospholipase C stimulation of protein kinase C (104,105).

Nitric oxide subserves the antithrombotic process by mechanisms other than its antiaggregatory and antiadhesive properties. The release products of "activated platelets" mediate changes in vasomotor tone in part through endothelium-dependent mechanisms. These factors, which in general result in vasodilatation if the endothelium is intact, have been shown to produce constriction in patients with atherosclerosis (76,106,107). Thus, the released platelet products can amplify the ischemic effects by causing constriction at sites of endothelial dysfunction. This adverse platelet-endothelium interaction is an important cause of vasoconstriction at sites of platelet aggregation.

Other actions. Although the major actions of endothelium-derived relaxing factor are related to its effect on vasomotion and platelet function, a number of other actions have been reported. Several studies have reported that endothelium-derived relaxing factor is cytoprotective and has an antiproliferative effect on smooth muscle cells (108-111). New interest has now been focused on the ability of endothelium-derived relaxing factor released from the myocardial cells to regulate local inotropy. Studies of septic shock suggest that vasodilation and decreased inotropy may result in hypotension because of the systemic release of large quantities of endothelium-derived relaxing factor. As a result of the
ubiquity of endothelium-derived relaxing factor, many new roles are likely to be discovered from future research.

Biologic Effects of Organic Nitrates

The actions of the organic nitrovasodilators parallel those of endothelium-derived relaxing factor. Major research efforts have focused on their ability to dilate coronary arteries and systemic arteries and veins and more recently on their ability to inhibit platelet aggregation.

Vascular smooth muscle. The major action of organic nitrates is the dilation of vascular smooth muscle in arteries and veins (112). The magnitude of this effect depends on the type of preparation studied and the nitrate preparation used. Nitroglycerin tends to exhibit a biphasic relaxation pattern, whereas isosorbide dinitrate and isosorbide-5-mononitrate (IS-5-MN) have no such biphasic response (113). Nitroglycerin has a greater sensitivity for veins than arteries (114). An in vitro study comparing the vasorelaxant effects of nitroglycerin, isosorbide dinitrate and isosorbide-5-mononitrate on isolated renal arteries and veins from rabbits suggested that the latter two were even more venoselective than nitroglycerin (115,116). Most in vitro studies have demonstrated that therapeutic doses of nitrates have only a small relaxing effect on peripheral arteries (117).

In vivo studies in animals, normal volunteers and patients with coronary artery disease, both with and without congestive heart failure have consistently shown that venous capacitance vessels have the highest sensitivity to nitrates (112). This venodilatation leads to a reduction in cardiac preload with a decrease in left ventricular volume and filling pressure that results in a decrease in oxygen consumption. The magnitude of the decrease in these variables is in the range 20% to 40%.

In patients with heart failure, sufficient arterial unloading occurs to actually increase cardiac output. The in vivo effect on compliance changes in large arteries may be greater than that predicted from in vitro studies (118).

Coronary artery dilation. A number of studies demonstrate that nitrates are potent dilators of both normal and atherosclerotic coronary arteries, a mechanism that may potentially increase blood flow to the myocardium. Feldman et al. (119) have shown that intracoronary doses of nitroglycerin can increase coronary artery diameter by 10% to 30%. Low doses of intravenous or sublingual nitroglycerin have also been shown to dilate coronary stenoses compared with placebo infusions (120,121). Brown et al. (122) subsequently demonstrated that ~75% of stenoses will dilate to some extent in response to nitroglycerin and suggested that this resulted from dilation of the normal portion of the artery in eccentric stenoses. Feldman et al. (123) also showed that dilation of collateral vessels was possible after nitrate administration. Reversal of distal or collateral vessel constriction, which may occur during exercise, may be another important mechanism by which nitroglycerin works (124,125). The extent to which epicardial coronary dilation relieves angina, however, remains controversial, although it probably plays some role.

One of the interesting aspects about organic nitrates is their relative inability to dilate small coronary arterioles in vitro. The response to nitroglycerin in vessels <100 µm can be brought out by incubation with thiols, implying that small vessels have an inability to convert nitroglycerin to its vasoactive metabolite. This remains speculative at present and has been nicely reviewed by Harrison and Bates (38). Despite this, however, nitrates may increase coronary flow by 25% to 50%, suggesting a mild effect on larger coronary resistance vessels (54,36,126). This effect is transient only and avoids the potentially deleterious coronary steal phenomenon that may be seen with potent arteriolar vasodilators.

Platelet effects. The effect of organic nitrates on platelet function has recently been reviewed (127). In vitro studies beginning in the late 1960s uniformly demonstrated an antiaggregatory effect of nitroglycerin on platelets (128,129). It was also known that nitroprusside can directly inhibit platelet aggregation and promote disaggregation (130,131).

As is the case for endothelium-derived relaxing factor, several groups have reported synergistic actions between the antiplatelet effects of nitroglycerin and nitroprusside with prostacyclin (132,133). In vitro studies using supraphysiologic concentrations have demonstrated that prostaglandin-mediated pathways may be important for the antiplatelet effects of nitrates (129,134,135).

Folts et al. (136) used an in vivo canine coronary model to demonstrate that platelet aggregation and dislodgment can be inhibited by nitroglycerin and that this effect may be mediated by cyclic GMP. Lam et al. (137) demonstrated that nitroglycerin can decrease platelet deposition in the common carotid arteries of balloon-injured pigs. Both studies demonstrated that the antiplatelet effect paralleled the hemodynamic changes induced by nitroglycerin. However, initial in vivo human studies failed to demonstrate an antiplatelet effect for the organic nitrates at physiologic concentrations (138). Stamler et al. (139) investigated the effect of intravenous nitroglycerin on platelets ex vivo. They found that in vivo infusions of nitroglycerin titrated to the hemodynamic response did not alter ADP-induced platelet aggregation. However, after they added N-acetylcysteine (a thiol donor) ex vivo, platelet inhibition was seen. Diodati et al. (140) used bedside impedance aggregometry before and after 45 min of nitroglycerin infusion and demonstrated a decrease in the area under the aggregation curve in response to ADP and thrombin, suggesting an antiplatelet effect. They also reported that if blood was left to stand for 30 min before analysis, no difference was noted between the control and nitroglycerin infusion (140). They believed that prolonged ex vivo experiments usually led to depletion of thiol groups and was a possible explanation for some of the previously negative results from studies using nitroglycerin (131,139,141). Another recently reported in vivo study (44) was able to demonstrate a good relation between platelet
cyclic GMP levels and coronary vasodilation to sublingual nitroglycerin in patients with and without nitrate tolerance. It is likely, but not conclusive, that nitrates have a beneficial antiplatelet effect in in vivo situations.

Endothelial Dysfunction: Possible Mechanisms

Endothelial vasodilator dysfunction has been demonstrated in vitro and in vivo in animal and human studies in a wide range of conditions, including hypercholesterolemia, atherosclerosis, hypertension, diabetes mellitus, heart failure and postischemic reperfusion. A detailed discussion is beyond the scope of this article, but several reviews are available (63). The mechanism of this vasodilator dysfunction is unclear, and because the actions of endothelium-derived relaxing factor are complex, abnormalities could occur at many sites of action: 1) impairment of endothelial membrane receptors that interact with agonists or physiologic stimuli to release nitric oxide; 2) diminished levels or impaired utilization of L-arginine, the substrate for nitric oxide synthesis; 3) reductions in nitric oxide synthase, the enzyme responsible for the conversion of L-arginine to nitric oxide; 4) impaired release of nitric oxide from the endothelium in its most active form (i.e., nitrosothiols); 5) enhanced degradation of nitric oxide by oxygen free radicals; 6) impaired transport from endothelium to smooth muscle cell (functional barrier); 7) impaired interaction of nitric oxide with guanylate cyclase, and subsequent increase in cyclic GMP; and 8) decrease in generalized smooth muscle cell sensitivity to vasodilators.

The best studied conditions associated with endothelial dysfunction are hypercholesterolemia and atherosclerosis. Hypercholesterolemia inhibits endothelium-dependent relaxation evoked by receptor-dependent stimuli, but this generally does not inhibit the direct relaxing effect of nitroglycerin. Studies in hypercholesterolemic porcine coronary arteries have demonstrated that the activity of the pertussis toxin-sensitive G protein-dependent receptor pathway is reduced in hypercholesterolemic endothelial cells (142, 143). This may be one of the mechanisms of endothelial dysfunction in the early stages of the atherosclerotic process (144).

The synthesis of nitric oxide involves the oxidation of a guanidino nitrogen on L-arginine by means of specific nitric oxide synthases. Several groups have suggested that a depletion of L-arginine is responsible for this endothelial dysfunction in hypercholesterolemia. It was shown in rabbit hind limbs that L-arginine administration potentiated the acetylcholine-induced increase in hind limb flow in rabbits with dietary hypercholesterolemia (145). Creager et al. (146, 147) also demonstrated that an infusion of L-arginine improved the forearm blood flow response to methacholine in hypercholesterolemic patients. Two similar studies have been performed in human coronary arteries. Drexler et al. (148) were able to demonstrate an improvement in the flow response to acetylcholine after L-arginine infusion but did not demonstrate any effect on large-vessel vasomotion. In contrast, Dubois-Rande et al. (149) showed an improvement in large-vessel response to acetylcholine after L-arginine infusion. Therefore, in certain situations, depletion or impaired utilization of L-arginine may contribute to the dysfunction seen in hypercholesterolemia; however, a deficiency of the substrate has not been proved by measurements of intracellular concentrations.

Rubanyi and Vanhoutte (9) have shown that superoxide anions can inactivate endothelium-derived relaxing factor. In states in which excessive free radicals are generated, an increase in oxidation of low density lipoproteins (LDLs) would also result. Oxidized LDL itself has been shown to be a potent inhibitor of endothelium-dependent relaxation through mechanisms that are transduction dependent (150, 151). Still others have suggested that free radicals interfere with the production of potent vasodilators and may lead to increased destruction of endothelium-derived relaxing factor (152). A recent study has shown that superoxide dismutase can attenuate the vasoconstricting effect of acetylcholine in the coronary arteries of patients with atherosclerosis (153), suggesting a role for oxygen free radicals in the inactivation of nitric oxide in vivo.

A defect in the vascular smooth muscle of resistance vessels in relation to the action of endothelium-derived relaxing factor has been suggested by two recent forearm studies. Creager et al. (146) demonstrated a decrease in the stimulated increase in flow in response to methacholine and nitroprusside in patients with hypercholesterolemia compared with control subjects. Calver and Vallance (154) also demonstrated a decrease in flow response to nitroprusside in patients with type I diabetes mellitus compared with control subjects. These studies did not determine whether the attenuated responses seen with nitroprusside are the result of augmented inactivation of nitric oxide from nitroprusside or are a generalized defect in smooth muscle cell function.

Other mechanisms of endothelial dysfunction include intimal thickening, which acts as a barrier to the diffusion of endothelium-derived relaxing factor (155) and the increased production of vasoconstrictor substances that offset the balance toward vasodilation. Endothelin may be one such factor. However, the role of these vasoconstrictors has not been well defined. Thus, even though the normal physiology of endothelium-derived relaxing factor has been extensively studied, the mechanisms involved in its failure are incompletely understood at this time.

Interactions Between Exogenous Nitrovasodilators and Endothelium-Derived Relaxing Factor

Extensive evidence is available to show that the chemical nature, mechanism of action and biologic actions of
endothelium-derived relaxing factor and the organic nitrates parallel each other. However, it is not known whether the administration of exogenous nitrates has an advantageous or potentially deleterious effect on the endothelium-dependent nitric oxide pathway or whether exogenous nitrates can replace the decreased activity of endogenous nitric oxide in various diseases. Moreover, it is unclear whether endothelial dysfunction affects the response of exogenous nitrates.

Effect of nitroglycerin tolerance on endothelium-dependent vasorelaxation. Potential adverse effects could result from prolonged nitrate therapy sufficient to induce nitrate tolerance. Thiol depletion could theoretically have an adverse effect on the intracellular activation of guanylate cyclase and the subsequent production of cyclic GMP. Some investigators have found cross-tolerance between nitroglycerin and endothelium-derived relaxing factor with regard to both relaxation and cyclic GMP increase (156,157). Rapoport et al. (157) for example, pretreated rat thoracic aortas to induce nitrate tolerance. They then demonstrated that there was attenuation of relaxation in response to nitroglycerin, nitroprusside and acetylcholine, with an associated decrease in the formation of cyclic GMP. Other investigators have found no cross-tolerance (158-160). Lawson et al. (161) studied rat aortic strips rendered nitrate tolerant and were able to demonstrate normal relaxation in response to acetylcholine. Thus, at this time there is inconclusive evidence to show whether nitrate tolerance has a significant physiologically adverse effect on endothelium-mediated events. The question, nonetheless, has important clinical ramifications.

Enhanced sensitivity to nitroglycerin in the setting of endothelial dysfunction. Most in vitro and in vivo studies have demonstrated retained or even augmented relaxant properties of nitrates when endothelial dysfunction is present (153). Moncada et al. (162) recently suggested that an enhanced sensitivity to nitrates may be present after removal of endothelial nitric oxide. They performed in vitro studies on isolated rat aortic rings and demonstrated that after endothelial denudation or inhibition of endothelium-derived relaxing factor, the potency of nitroglycerin and nitroprusside as relaxing agents was significantly enhanced. They also demonstrated an enhanced hypotensive response to nitroglycerin in rats given nitric oxide synthase inhibitors compared with rats without endothelium-derived relaxing factor inhibition. These experiments suggested that a supersensitivity to nitrovasodilators at the level of soluble guanylate cyclase was induced by the removal or inhibition of endothelial influences. Holland et al. (163) suggested that the endothelium is known to produce reactive oxidative metabolites that can scavenge nitric oxide derived from exogenous vasodilator drugs and that its removal or inhibition might result in an enhanced response to exogenous nitrates as an alternate mechanism. Either way, this has important clinical implications.

Nitroglycerin as replacement for endogenous nitric oxide. The short-term administration of nitroglycerin has been shown to prevent endothelium-dependent constriction in response to exercise in patients with coronary stenoses in vessels presumably deficient in endogenous nitric oxide. The vasomotor response of exercise at sites of stenoses has been shown to be vasoconstriction (84). This vasoconstriction paralleled changes seen in response to acetylcholine and have been interpreted to be due to endothelial dysfunction. Gage et al. (164) also studied the effect of exercise on vasomotion at stenotic sites in patients with angina. They demonstrated a decrease in area of 71% at stenotic sites in response to exercise; nitroglycerin subsequently increased the area to 12% above control levels. In patients given nitroglycerin before exercise, there was no decrease in area at the stenotic site as a result of exercise. Thus, prevention of sympathetically mediated, endothelium-dependent vasoconstriction may be an important anti-ischemic mechanism of nitroglycerin. Other evidence of nitroglycerin as a replacement for endogenous nitric oxide is lacking in humans at this time, but the use of nitrates as surrogates for diminished intrinsic nitric oxide would be very appealing. However, improper timing of nitrate administration might deplete thiol stores, negating any potential beneficial effects of administered nitrates.

Conclusions

The organic and inorganic nitrovasodilators share many common features with the recently discovered endothelium-derived relaxing factor. The one major difference may be the lack of effect of organic nitrates in the coronary microcirculation. Both endothelium-derived relaxing factor and nitrovasodilators have similar biologic effects as a result of their similar, final common pathway. They predominantly relax vascular smooth muscle in most species and tissue beds tested. Recent data have demonstrated the potent antiplatelet effect of endothelium-derived relaxing factor and exogenous nitrates, which may act in concert with prostacyclin.

The action of the exogenous nitrovasodilators may be enhanced in patients with dysfunctional endothelium, and, conversely, nitrate tolerance may impair endothelium-dependent processes. Much needs to be learned about the potentially important interaction between exogenous nitrates and the "endogenous nitroglycerin," endothelium-derived relaxing factor. Such insights might enable a more logical use of our oldest and most prescribed antianginal medication.

References

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