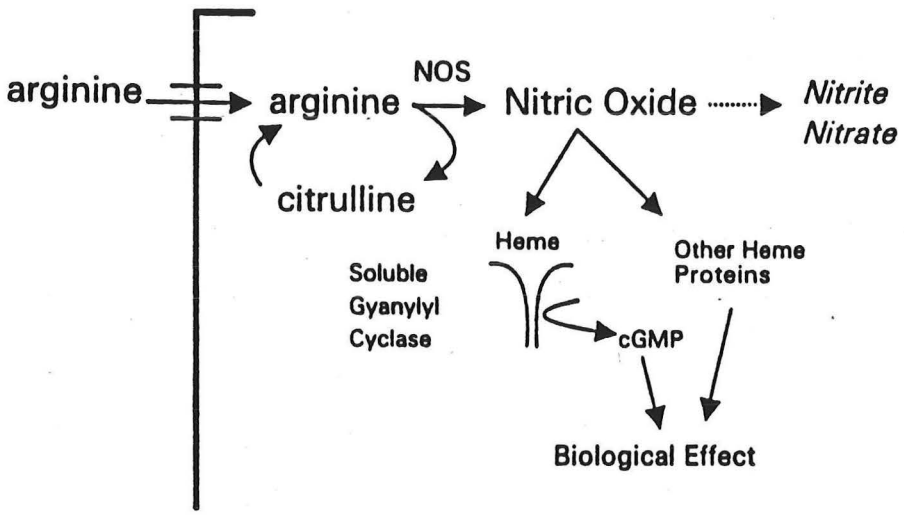
University of Texas
Southwestern Medical Center
at Dallas

Nitric Oxide



Robert Alan Star, MD
Medical Grand Rounds
March 25, 1993

INTRODUCTION

Ten years ago, nitric oxide or NO, an environmental pollutant found in cigarette smoke and smog was suspected to be a carcinogen, destroy ozone, and produce acid rain. However, over the last 5-10 years, research in such diverse fields as immunology, toxicology, and vascular physiology has converged to show that this highly toxic molecule has a myriad of actions throughout the body that range from blood pressure regulation to antimicrobial defense to intracellular signal transduction (1). This startlingly simple molecule has revised the scientific understanding of how cells communicate and defend themselves. As a result, nitric oxide was named "molecule of the year" in 1992 by Science. Nitric oxide, also known as endothelium derived relaxing factor or EDRF, is not to be confused with nitrous oxide, or N_2O, otherwise known as laughing gas. Humphry Davy, who discovered analgesic properties of N_2O in the late 18th century almost died when he inhaled nitric oxide (2). This grand rounds will focus on vascular effects of nitric oxide and the therapeutic agents which modulate this pathway.

PHYSIOLOGY

Nitric oxide is a colorless, lipophilic gas that is slightly soluble in water. It is highly chemically reactive because it possesses an extra electron. Because it is a free radical, nitric oxide has a short half life of 6-30 seconds because of rapid oxidation to nitrite and nitrate. This partially accounts for the difficulty early researchers had in its discovery. The short half life indicates that the effects of nitric oxide are limited a local site of action of approximately 200-600 μM. Nitric oxide can react with proteins, forming nitroso adducts, which may mediate some of the biological activities of nitric oxide (3-5).

Nitric Oxide Signal Cascade. Figure 1 shows a schematic pathway of nitric oxide signalling. Nitric oxide is synthesized from the guanidine nitrogen atom of L-arginine by nitric oxide synthase. L-citrulline is also produced as an inactive end product. Nitric oxide diffuses within the cell or to an adjacent cell where it stimulates soluble guanylyl cyclase, or other heme containing proteins. The resultant increase in cyclic GMP in the target cell produces the physiologic effect. For example, in smooth muscle cells, cyclic GMP decreases cell calcium which leads to relaxation of the smooth muscle cell and thus vasodilation. The arginine precursor either enters the cells via an arginine transporter or can be recycled from citrulline.

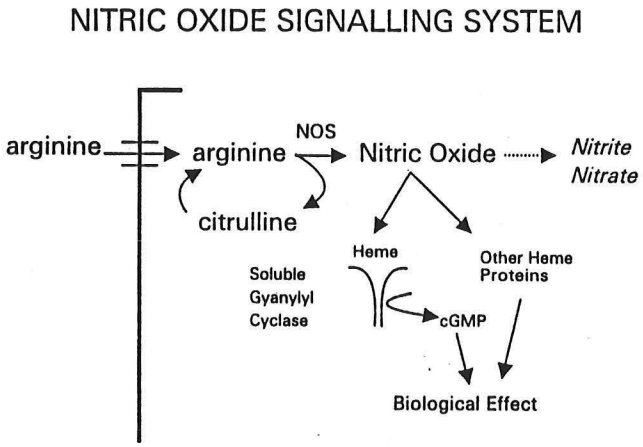


Figure 1

Modes of Action. Nitric oxide has several different modes of action [Figure 2]. Nitric oxide mediates communication between adjacent cells, signals inside cells, and

is cytotoxic. The first mode accounts for its vasodilatory properties which it signals from endothelium to underlying vascular smooth muscle cells. Nitric oxide has recently been shown to modulate tubular glomerular feedback: nitric oxide produced in the macula densa diffuses to the afferent arterial where it opposes the vasoconstrictive signal which mediates tubular glomerular feedback (6). As a consequence, nitric oxide plays important roles in etiology of systemic hypertension, pulmonary hypertension, and ischemia. Nitric oxide can also function as a neurotransmitter and may play an important role in long term potentiation and memory (1).

Physiologic Actions of Nitric Oxide

Communication between adjacent cells
Intracellular communication
Cytotoxic agent

Figure 2

Nitric oxide signals within cells to inhibit platelet aggregation and insulin release. Nitric oxide in the mesenteric neuronal plexus controls the relaxation of sphincters along the GI tract and mediates propulsion of luminal contents in the small and large intestine. At high concentrations, nitric oxide is a cytotoxic agent that kills cells and bacteria. These actions are important in the prevention of bacterial and chronic infections and may be involved in the pathogenesis of vascular strokes and several degenerative neurologic diseases (1).

Nitric Oxide Synthases. Nitric oxide synthase is a family of enzymes which currently contains three isoforms (7,8) [Figure 3]. The first member of this family was purified from rat cerebellum (9). Sequencing of the cDNA revealed recognition sites for calmodulin, NADPH, FAD, FMN, and several phosphorylation sites indicating that the synthase could be regulated by many different factors. In situ hybridization revealed prominent labeling in cerebellum, hence its original name, cerebellar nitric oxide synthase.

Nitric Oxide Synthase Isoforms

		Regulation	
Name	Location	Acute	Chronic
Endothelial	endothelial cell	Ca^{++}, O_2	flow
Brain	widespread	Ca^{++}	
Inducible	macrophage		cytokines
	widespread		steroids

Figure 3

PCR based cloning techniques were used to isolate two other members of this family from macrophages (10,11) and endothelial cells (12-15). All three different forms have unique tissue distributions and patterns of regulation. The endothelial form is found in endothelial cells where it is regulated by acute changes in intracellular calcium/calmodulin and oxygen, as well as chronic changes in shear stress. The brain form is widely distributed throughout the body in addition to the cerebellum where it was originally described. This form is acutely regulated by cell calcium. The macrophage form is also expressed in vascular smooth muscle cells, liver, kidney tubules, and interstitial cells. It does not seem to be regulated acutely but rather its expression is induced by cytokines (tumor necrosis factor B and interferon gamma), and its expression is inhibited by steroids. Because of the widespread distribution of

this family of enzymes, pharmacologic modulation of specific isoforms or functions of the pathway will require more specific inhibitors than currently available.

Nitric Oxide Receptors. The main target for nitric oxide is soluble guanylyl cyclase [Figure 4]. Soluble guanylyl cyclases contain heme which is the receptor for nitric oxide. Binding of nitric oxide to the heme group induces a conformational change which displaces the iron out of the plane of the porphyrin ring thus activating the soluble guanylyl cyclase (16). Other proteins containing heme can react with nitric oxide (aconitase and cytochromes of the mitochondrial electron transport chain), which may account for its inhibition of insulin release and cytotoxicity (17,18).

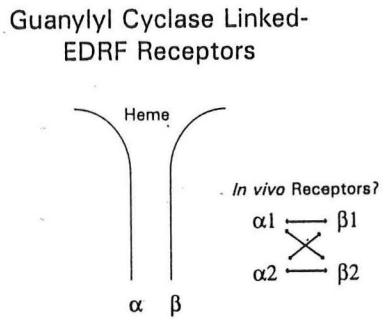


Figure 4

Soluble guanylyl cyclases are heterodimers comprised of two different subunits called α and ß. Two α and two ß subunits have been cloned to date (19-21). α1 and ß1 are widely distributed throughout the body while β2 is expressed mainly in the kidney and liver. The distribution of α2 is unknown. We have shown that the renal blood vessels (comprised of endothelial and vascular smooth muscle cells) contain mRNA for the α1, β1 and α2 receptor while the cortical collecting duct, where nitric oxide inhibits sodium transport, expresses mRNA for α1 and β2 (22). The physiologic significance of these different isoforms is unknown. ß2 has a potential isoprenylation site, which could allow ß2 to be associated with membranes. Preliminary evidence suggests that β2 might act as a dominant negative since it may be less active when combined with α1 than β1 (23).

Inactivation of Nitric Oxide. Nitric oxide can be irreversibly inactivated by reaction with hemoglobin in the blood vessel lumen (both oxygenated or deoxygenated forms), superoxide radical found within a blood vessel wall, or oxygen in free solution. Combination with oxygen produces nitric acid and nitrous acid, resulting in the stable end products nitrite and nitrate, which are easily detected.

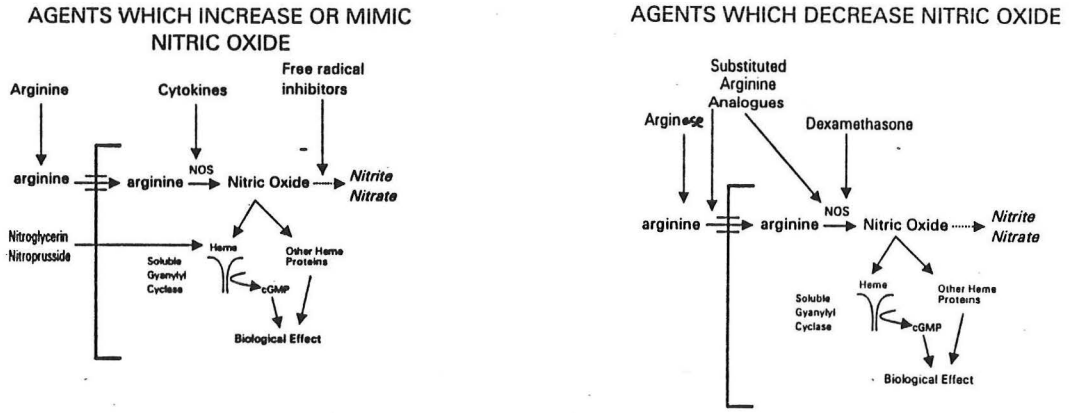


Figure 5

Pharmacologic Modulation. The nitric oxide signal could potentially be increased at several different sites [Figure 5]. Transport of arginine into the cell is increased by exogenous arginine. Nitric oxide production by the macrophage nitric oxide synthase can be induced by cytokines. The half life of nitric oxide could be prolonged by depleting free radicals (i.e., superoxide) with superoxide dismutase (24). The nitric oxide signal could be by mimicked by nitroprusside or nitroglycerin which are either chemically or metabolically converted to nitric oxide. There are several agents which are known to decrease the nitric oxide signal [Figure 5]. Substituted arginine analogs (L-NMMA, L-NAME) prevent arginine from getting into cells and directly inhibit arginine from interacting with the nitric oxide synthase. Steroids prevent induction of the macrophage form of nitric oxide synthase.

An endogenous inhibitor, asymmetrical dimethylarginine or ADMA, can be detected in normal human subjects, and accumulates in patients with chronic renal failure (25,26). There role in the pathogenesis of hypertension in patients with chronic renal failure has been the subject of a recent grand rounds (27).

Vascular Tone. Vascular tone is regulated by opposing factors which mediate vasodilation and vasoconstriction [reviewed in (28,29)]. The balance between these factors determines the level of arterial tone on a moment-to-moment basis. Figure 6 shows a model of nitric oxide production in the arterial wall. Nitric oxide synthesis is increased by acetylcholine, bradykinin, angiotensin II, 5-hydroxytryptamine, ergotamine, adenine nucleotides, and thrombin. Vasodilatory neurotransmitters, such as bradykinin or acetylcholine, stimulate calcium entry in the endothelial cell which increases cell calcium and activates the endothelial form of nitric oxide synthase by a calmodulin dependent mechanism. Nitric oxide diffuses into the adjacent vascular smooth muscle where it binds heme and thus activates soluble guanylyl cyclase. Production of cyclic GMP decreases cell calcium which mediates the relaxation of the smooth muscle cell (30).

Vascular Nitric Oxide Signal Transduction Pathway

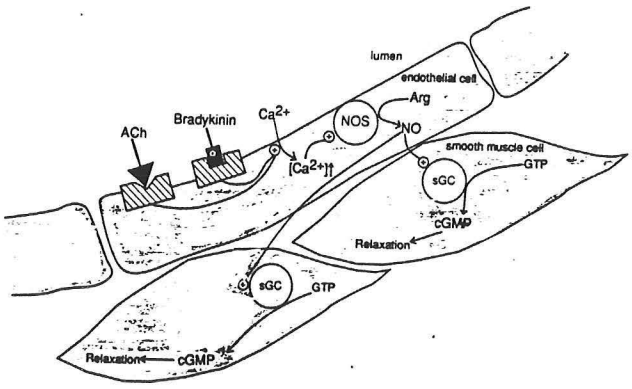


Figure 6

The endothelial cell also contains a soluble guanylyl cyclase. We have shown that it consists of a $\alpha 1/\beta 1$ subunit, and functions to increase endothelial cell calcium (31). This positive feedback may allow the endothelial cell to produce a burst of nitric oxide, or respond to nitric oxide produced in other cells. In addition, both endothelial and vascular smooth muscle cells contain the inducible form of nitric oxide synthase which when activated, produces approximately a thousand-fold greater amounts of nitric oxide than that produced by the endothelial nitric oxide synthase (7,32,33) These synthases are important in septic shock (see below). Arginine enters the endothelial cells via a specific transporter. Endothelial cells studied in tissue culture

and in vivo maintain their cell arginine level despite the continuous release of nitric oxide in part by recycling citrulline to arginine (34,35). However, as discussed below, data from arginine infusions in human subjects suggests that arginine could be rate limiting. Thus, extrapolation from animal studies must be done cautiously.

Clinical Assessment of Nitric Oxide Action. Activity of the nitric oxide signaling system [Figure 7] can be noninvasively assessed by the response of systemic blood pressure, blood citrulline and urinary nitrite and nitrate to agents which stimulate (acetylcholine) or inhibit (L-NMMA) nitric oxide production. Some regional beds have less basal nitric oxide production (36), so global measurements (blood pressure, serum and urine nitrite concentrations) may not correlate with the response in a specific organ bed. Effects on the cardiac circulation can be assessed by measurements of epicardial blood vessel diameter, and coronary blood flow during cardiac catheterization. Changes in the epicardial vessel diameter indicate local effects on the conduit blood vessel, while changes in organ blood flow indicate effects at the level of resistance vessels. Most regulation of blood flow occurs in the small resistance blood vessels; unless there is significant stenosis (i.e., greater than 90% occlusion), there is little pressure drop across the conduit artery.

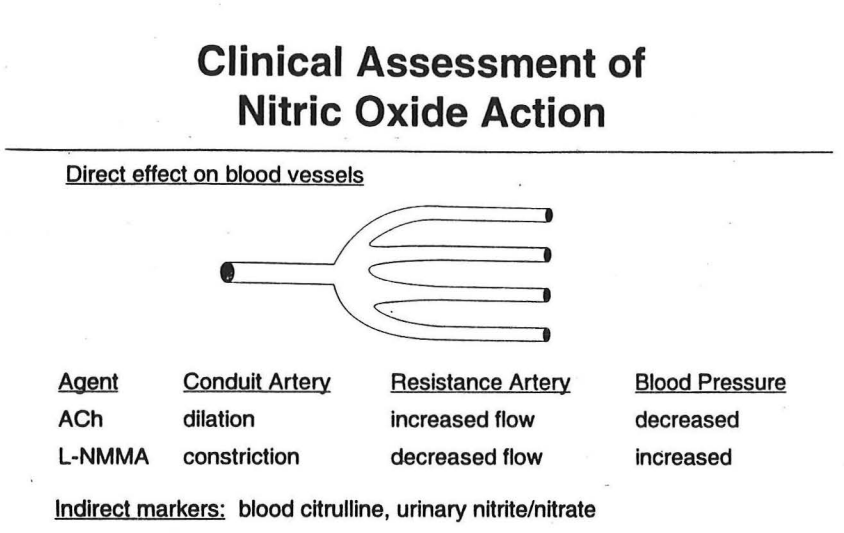


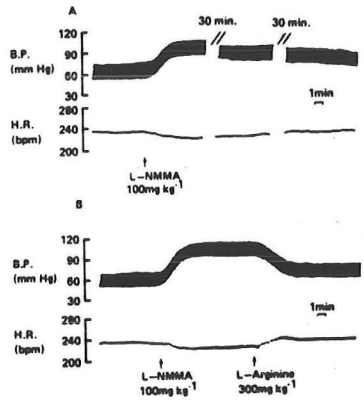
Figure 7

DEFICIENCY OR INCREASED DESTRUCTION OF NITRIC OXIDE

Nitric oxide deficiency and increased destruction of nitric oxide are difficult to differentiate clinically, so they will be discussed together. There is evidence for this defect(s) in systemic hypertension, coronary artery disease, pulmonary hypertension associated with COPD and ARDS (37-39), and GI motility disorders (40).

Regulation of resting blood pressure. A number of recent studies in laboratory animals and human subjects have shown that nitric oxide formed from arginine by the vascular endothelium is an important determinant of resting peripheral vascular resistance and blood pressure. In anesthetized rabbits, inhibition of nitric oxide production with substituted arginine analogues (L-NMMA or L-NMA) acutely increased blood pressure and caused a reflex bradycardia [Figure 8]. The hypertensive effects were reversed by L-arginine but not by the inactive isomer D-arginine (41). Similar

Effect of L-NMMA on Blood Pressure in Rabbits

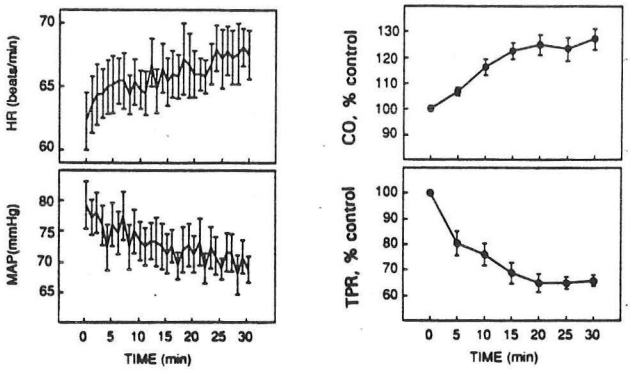


Rees, Palmer, Moncada, PNAS, 1989

Figure 8

studies have not been reported in humans perhaps because of the risk of myocardial ischemia (see below).

Effect of Acute L-Arginine in Normotensive Subjects



Hishikawa, et. al., Jpn. Heart J., 1992

Figure 9

In normotensive subjects, L-arginine infusion decreased total peripheral resistance, causing hypotension and a reflex tachycardia [Figure 9] (42,43). Plasma concentrations of cycle GMP, citrulline, and urinary nitrite and nitrate were significantly increased, confirming the rise in overall nitric oxide production. The use of L-arginine as an antihypertensive agent is discussed below. The site of this effect of these agents (vascular wall vs. brain) is uncertain, since nitric oxide synthase is also present in the peripheral and central nervous system where it functions as a neurotransmitter to inhibit sympathetic outflow (27,44).

Hypertension. The endothelium may play a causative role in the development of hypertension. The endothelium is also a target of the hypertension since it is exposed to mechanical shear forces and vasoactive hormones which increase peripheral vascular resistance. Alterations in vascular endothelium function may lead to development of decreased organ profusion manifested by myocardial infarction and stroke. Thus, because of the critical role of nitric oxide in maintaining basal blood pressure, it has been proposed that nitric oxide pathway is abnormal in patients with hypertension (45,46).

Hypertension: Rat Models. Data from rat models of hypertension indicate a close tie between nitric oxide deficiency and hypertension. First, rats with experimentally-induced deficiency of nitric oxide produced by chronic nitric oxide synthesis inhibition with L-NAME have a syndrome which mimics malignant hypertensive nephrosclerosis (i.e., severe hypertension, glomerulosclerosis, and massive proteinuria) (47). Second, a strain of rats which become hypertensive on a high salt diet is nitric oxide

Dahl/Rapp Salt Sensitive Rats Are Nitric Oxide Deficient

	NO production	Blood Pressure
Low salt diet	normal	normal
High salt diet	deficient	hypertensive
+ arginine	normal	normal

Figure 10

deficient [Figure 10] (48,49). The Dahl/Rapp strains of salt sensitive and salt resistant rats were inbred to either develop, or not develop, hypertension on a high salt diet. In response to the high salt diet, the salt resistant rats increased production of nitric oxide, and remained normotensive. In contrast, salt resistant rats did not increase production of nitric oxide, and developed morphologic features of malignant hypertension. Provision of arginine to these rats prevented the hypertension and the development of chronic renal failure (49). Nitric oxide production increased. Preliminary studies to determine the site of the metabolic derangement have shown that dexamethasone prevents the antihypertensive effect of arginine (45). Thus, the response to arginine may be mediated by an inducible form of nitric oxide synthase. Arginine also prevents hypertension in other animal model of hypertension (50).

Furthermore, nitric oxide may play role in the adaptation to increased dietary salt intake. Rats placed on a high salt diet have increased urinary nitrite and nitrate excretion (51). The nitric oxide plays a prominent role in the regulation of renal hemodynamics, and also may have a direct effect to inhibit tubular sodium transport in the proximal tubule and cortical collecting duct (52,53).

Hypertension: Human Studies. More recently, it has been demonstrated that the endothelial nitric oxide pathway is abnormal in patients with hypertension. First, patients with untreated essential hypertension have an abnormal response to infusion of L-NMMA (54) [Figure 11]. The nitric oxide synthase inhibitor L-NMMA was infused into the brachial artery and forearm blood flow measured. Normotensive control patients had an equal response to intraarterial norepinephrine and intraarterial L-NMA. In contrast, hypertensive patients had a normal response to norepinephrine but a diminished response to L-NMMA. These studies suggest that the basal release/action of nitric oxide is abnormal in patients with untreated hypertension.

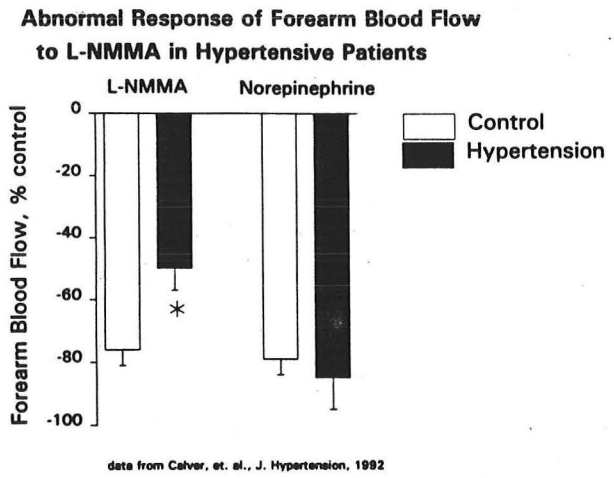


Figure 11

Second, hypertensive patients have an abnormal response of forearm blood flow and vascular resistance to endothelium-mediated vasodilators such as acetylcholine (55,56) [Figure 12]. The subjects were treated with aspirin to block protacyclin production and with the alpha adrenergic blocker phentolamine. The vascular effects of acetylcholine were independent of prostaglandins and adrenergic neurotransmitters and therefore likely to be mediated by nitric oxide. In contrast, the response to the endothelium-independent vasodilation nitroprusside was normal. Thus, acetylcholine-stimulated nitric oxide release/action is also abnormal in hypertensive patients, indicating endothelial dysfunction. The endothelial dysfunction occurs at the level of the resistance arteries, because forearm *blood flow* was affected (55-57). These results are striking because this part of the circulation rarely develops

hypertensive vascular disease. As described below, the coronary circulation, a known target of hypertension, seems to exhibit more severe endothelial dysfunction.

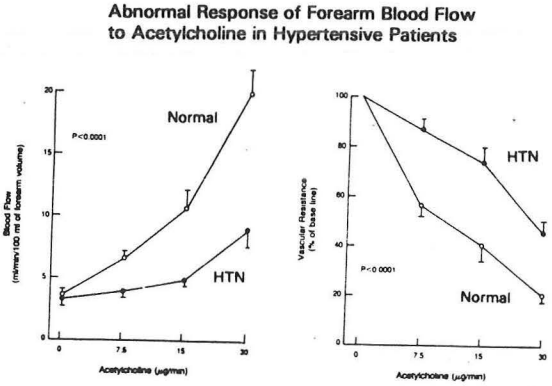
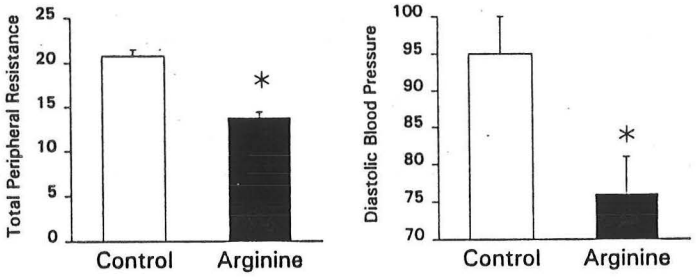


Figure 12

Arginine as a Anti-Hypertensive Agent in Hypertensive Patients



data from Hishikawa, J. Cardiovasc. Pharm., 1992

Figure 13

Several studies have shown that intravenous arginine infusion lowers blood pressure in patients with both essential hypertension and secondary causes of hypertension (42,43,58) [Figure 13]. Acute infusion of arginine decreased total peripheral resistance, decreased mean arterial pressure, and increased heart rate and cardiac output. Arginine also releases histamine, insulin, glucagon and prolactin; however, similar results were found in patients treated with chlorpheniramine, a H1-antagonist (59). The role of the other hormones in the response to arginine is unknown (43). The studies also found an increase in plasma citrulline and urinary nitrate and nitrite, markers of increased nitric oxide production. Blood pressure decreased to a greater extent in hypertensive than normotensive patients (42).

These dramatic results indicate that arginine might be useful for treating accelerated or malignant hypertension. Arginine infusion is not without risk since extrapolation can cause local tissue necrosis (60). The effects of chronic arginine therapy in humans have not been reported. In addition to the vascular effect, arginine may alter blood pressure by direct effects on sodium excretion. Stoos and Garvin have shown that nitric oxide decreases short circuit current, a marker for sodium transport, in cultured cortical collecting duct cells (53).

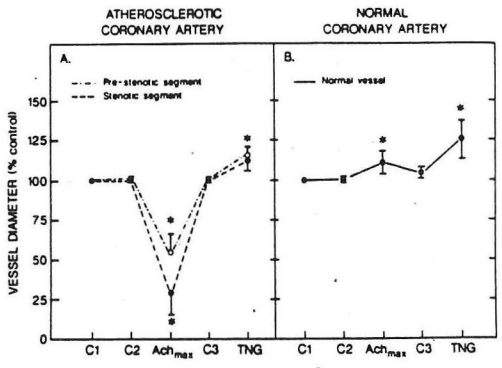
In summary, hypertensive patients have evidence of endothelial dysfunction. Whether the endothelial dysfunction is the cause or consequence of systemic hypertension is not yet clear. A primary deficiency of nitric oxide would lead to vasoconstriction and thus a rise in blood pressure. On the other hand, an increase in vasoconstrictor tone could cause endothelial dysfunction. In animals models, the endothelial dysfunction associated with acute hypertension can be prevented by initiation of antihypertensive therapy, suggesting that the defective nitric oxide release is a consequence of the systemic hypertension. In humans, the opposite may occur.

Endothelium-dependent forearm vasodilation is reduced in normotensive patients with a family history of hypertension (61). While this is a small study involving only 13 hypertensive patients and 13 normotensive controls, it raises the possibility that endothelial dysfunction can precede the appearance of hypertension and thus that endothelial dysfunction might play a causal role in the pathogenesis of essential hypertension (61).

Ischemia. The initial studies of Gardiner showed that basal release of nitric oxide is an important determinant of organ blood flow (36), which immediately suggested that abnormal regulation of the nitric oxide pathway could be an important cause of ischemia, especially myocardial ischemia. Indeed, L-NMMA decreases coronary blood flow in animals (62,63), suggesting that basal nitric oxide production is important for regulating coronary blood flow. Subsequent studies have proposed several mechanisms by which nitric oxide deficiency could produce ischemia: decreased vasodilation or paradoxical vasoconstriction in response to vasodilators, enhanced response to vasoconstrictors, increased platelet aggregation and adhesion, or proliferation of vascular smooth muscle cells (64-66).

Decreased vasodilation or paradoxical vasoconstriction in response to endothelium-dependent vasodilators. As described above, hypertensive patients have a decreased vasodilation in response to acetylcholine. Paradoxical vasoconstriction induced by acetylcholine was first described in the coronary circulation by Ludmer in patients with both mild (<20% occlusion) and in severe (>50% occlusion) coronary artery disease [Figure 14] (67). All coronary artery segments exhibited normal vasodilation in response to nitroprusside, indicating preservation of vascular smooth muscle relaxation.

Paradoxical Response of Coronary Artery to Intracoronary Acetylcholine

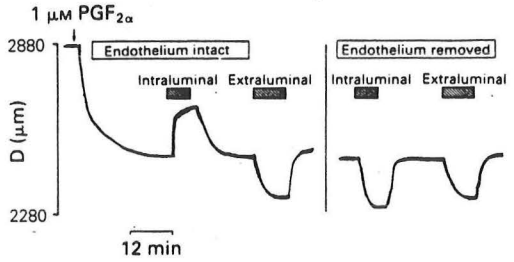


Ludmer, et. al., N. Engl. J. Med., 1986

Figure 14

An explanation for this paradoxical response to acetylcholine is shown in Figure 15 (68). As described above, acetylcholine dilates normal blood vessels by releasing nitric oxide from the endothelial cells. By contrast, if the endothelium is damaged or removed experimentally, acetylcholine may paradoxically constrict blood vessels because of a direct effect on the exposed vascular smooth muscle. Therefore, a paradoxical effect of acetylcholine has been taken as evidence of endothelial dysfunction.

Paradoxical Response to Endothelium-Dependent Vasodilators in the Absence of Endothelium

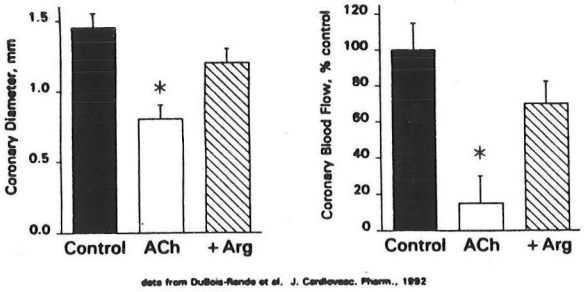


Brusse and Bassenge, Br. J. Pharm., 1992

Figure 15

Subsequent studies have confirmed these results and shown that coronary blood flow regulation is also impaired in patients with mild or early atherosclerosis (69-71) [Figure 16]. Acetylcholine decreased blood flow out of proportion to change in large blood vessel diameter. The subnormal or paradoxical response of coronary blood flow to the vasodilator acetylcholine indicates a concurrent defect in the endothelium of small resistance blood vessels, i.e., in microvascular disease that would not be demonstrable on coronary angiography. This is surprising, since this portion of the circulation is morphologically normal. Also, some studies of patients with coronary artery disease have shown endothelial dysfunction in coronary arteries that are angiographically normal. These microvessels are nevertheless exposed to high levels of circulating lipids and cholesterol; oxidized LDL inhibits acetylcholine-induced vasodilation in isolated rings of pig coronary arteries (72). The mechanism of the endothelial dysfunction is uncertain, but most studies indicate impaired nitric oxide release or action. In diabetes, advanced glycosylation products which accumulate on tissue proteins, destroys nitric oxide (73). Inhibition of the advanced glycosylation with aminoguanidine improves the defect in vasodilation.

Response of LAD Coronary Artery To Intracoronary Acetylcholine (ACh) in Hypertensive Patients



data from DuBois-Rande et al. J. Cardiovasc. Pharm., 1992

Figure 16

Endothelial dysfunction is improved by intracoronary arginine infusions of arginine; the improved endothelium-dependent relaxation both in the conduit and resistance blood vessels of patients with mild coronary artery disease (71) [Figure 16]. Similar results have been obtained in hypercholesterolemic patients (74). This raises the possibility that acute or chronic L-arginine could be used clinically to treat coronary ischemia.

Platelet aggregation. A second mechanism by which nitric oxide deficiency could cause ischemia is shown in Figure 17. Aggregated platelets produce nitric oxide, which functions to inhibit platelet aggregation (64). Human platelets also contain an arginine/nitric oxide pathway (75). Thus, platelets have a short-loop feedback mechanism which uses nitric oxide to prevent and reverse aggregation.

In addition, the platelet-endothelial cell interaction provides a second feedback loop to prevent platelet aggregation [Figure 17] (64). Aggregating platelets produce ADP, which stimulates endothelial nitric oxide production. The nitric oxide acts locally e16to prevent platelet aggregation near the vascular wall; an effect on platelets in the

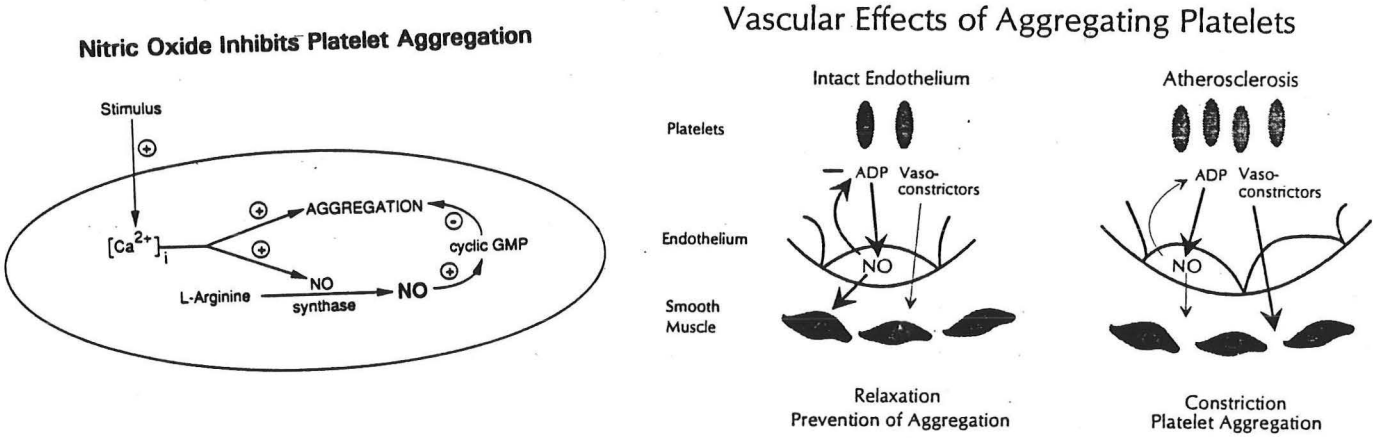


Figure 17

center of the vessel is unlikely since nitric oxide is destroyed by hemoglobin. The endothelial nitric oxide also overrides the effect of vasoconstrictors (thromboxane A_2 and serotonin) also released from platelets. The net result is vasodilation and prevention of platelet aggregation. In atherosclerosis, endothelial dysfunction with decreased nitric oxide release/action impairs this protective mechanism, favoring vasoconstriction and platelet aggregation and adhesion. This may promote vasoconstriction, thrombus formation and ischemia.

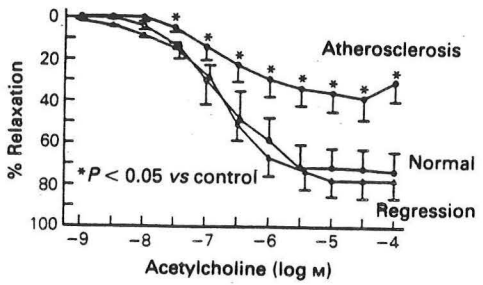
This scheme has been demonstrated experimentally; aggregated platelets dilate normal arteries, but constrict arteries from atherosclerotic animals (64). Furthermore, exogenous serotonin induces vasodilation in normal coronary arteries, but induces vasoconstriction or vasospasm in patients with atherosclerosis (76,77).

Implications of endothelial dysfunction. Could this endothelial dysfunction contribute to clinical syndromes of angina? While there is no hard evidence at the present time, there has been much speculation that this may occur. First, endothelial dysfunction in the coronary or peripheral circulation has been found in patients with diseases which are risk factors for ischemic heart disease [hypertension (54-56), atherosclerosis (67,69,70,78,79), hypercholesterolemia (74,80), diabetes (81), and patients with a puzzling syndrome of chest pain and normal coronary arteries (Syndrome X) (82-85)].

Second, a recent multivariate analysis has found that impaired coronary blood flow response to acetylcholine was associated with the presence of atherosclerosis, hypertension, hypercholesterolemia, age >50 and total number of coronary risk factors (79). Interestingly, smoking, male sex, and positive family history were not associated with an impaired response to acetylcholine nor responses to papaverine, an endothelium-independent vasodilator. Unfortunately, this study did not comment on the association of clinical angina and this abnormal coronary blood flow response. The similarity of risk factors for coronary artery disease and endothelial dysfunction suggests that endothelium dysfunction may be a marker for coronary artery disease. However, at the present time, the association between ischemia and nitric oxide deficiency must be regarded as speculative, but deserving further detailed study.

Correction of risk factors. Can correction of risk factors reverse endothelial dysfunction? The answer, in studies of experimental animals, is yes. Monkeys with diet-induced atherosclerosis have paradoxical responses to acetylcholine of coronary artery diameter and coronary blood flow [Figure 18] (86,87). Reversion of the atherosclerosis by dietary lowering of the serum cholesterol completely restored large vessel endothelial function to normal. Whether this occurs in humans is unknown.

Restoration of Endothelial-Dependent Relaxation By Dietary Treatment of Atherosclerosis



Harrison, et.al., J. Clin. Invest., 1987

Figure 18

Action of Nitroglycerin. Organic nitrates (nitroglycerin) are used to treat angina, myocardial infarction, and some forms of congestive heart failure. This topic has been the subject of a recent Grand Rounds (88). The mechanism of action of nitroglycerin is shown in [Figure 19]. Nitroglycerin enters the cell where it is biotransformed by a thiol-dependent enzyme system into nitric oxide or a closely related compound. In contrast, vasodilators such as nitroprusside or the newly-developed SIN-1 undergo a chemical conversion in solution to nitric oxide. This difference in mechanism of action becomes critical in evaluation of the mechanism of tolerance to nitroglycerin, a well known clinical phenomenon.

Mechanism of Action of Nitrovasodilators

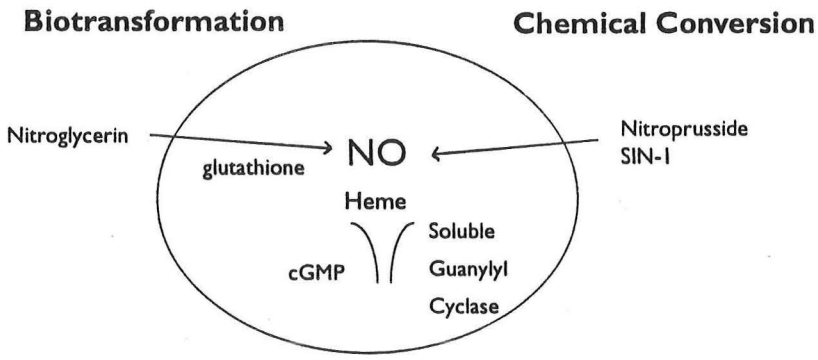


Figure 19

Prolonged administration of organic nitrates generally induces a state of tolerance (88). The mechanisms of tolerance include the initiation of powerful conterregulatory reflexes, and alteration in the target tissue itself, so that nitroglycerin loses its therapeutic efficacy. The mechanism of tolerance is not completely understood, but *in vitro* studies have shown that nitroglycerin rapidly depletes sulfhydryl groups needed for the biotransformation to nitric oxide (89). Studies by May, Popma, Black, Schaefer, Lee, Levine, and Hillis have shown that administration of N-acetylcysteine reverses the tolerance to nitroprusside (90).

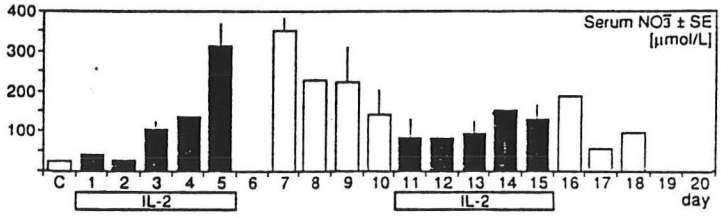
NITRIC OXIDE EXCESS

Nitric oxide excess is seen during IL-2 therapy (91), septic shock, uremic bleeding, hypotension during dialysis (27), and stroke syndromes (1,92).

Septic Shock. Gamma interferon and lipopolysaccharide induce formation of the macrophage or inducible nitric oxide synthase and increase urinary excretion of nitrite and nitrate in rats and mice (7,10,11). The latter is inhibited by L-NMMA. Immunologically activated macrophages produce exceedingly high concentrations of nitric oxide that do not act through cyclic GMP but instead are directly cytotoxic and immunogenic. Nitric oxide combines with superoxide to form peroxynitrite which induces DNA damage and mutation (93,94). Nitric oxide also inactivates iron containing proteins, including enzymes responsible for mitochondrial respiration and aconitase activity (95-99) so that cells cannot grow or divide. This high-output macrophage system seems to have evolved as an defense against intracellular organisms. Activated cultured macrophages can inhibit the growth of mycobacteria, pathogenic fungi, and parasites (17). For example, Alspraugh and Granger have shown that the growth of cryptococcus neoformans is inhibited in a cell-free system by nitric oxide gas (100). Local inhibition of nitric oxide synthesis with L-NMMA prevented clearing of Leishmania infections in murine footpads (101), suggesting that nitric oxide is important *in vivo*. LPS, endotoxin, and various cytokines also increase nitric oxide production in vascular endothelium, vascular smooth muscle, and other tissues by induction of the macrophage from of nitric oxide synthase (33,102-104). This induction is inhibited by dexamethasone (105).

The macrophage and vascular pathways have been conclusively demonstrated in rodent and murine tissues. Demonstration of an inducible nitric oxide synthase in humans has been very difficult, perhaps because the demands imposed by upright posture have dictated that the inducible system is very tightly controlled. However, patients receiving IL-2 cancer chemotherapy for renal cell carcinoma or malignant myeloma have increased serum

Evidence for Inducible Nitric Oxide Synthesis in Patients Receiving Interleukin-2 Therapy



Hibbs, et.al., J. Clin. Invest., 1992

Figure 20

and urine nitrite and nitrate produced from labeled arginine (91) [Figure 20]. This provides the best evidence that an inducible NOS is present in humans.

Nitric oxide plays a double edged role during septic shock [Figure 21] (106,107). Nitric oxide is beneficial because it is antibacterial. However, nitric oxide also has deleterious effects, including sustained vasodilation, hypotension, and hyporeactivity to vasoconstrictor agents (104,108). In animal studies, the progressive hypotension is mediated by enhanced formation of nitric oxide by an inducible nitric oxide synthase. This suggests that inhibition of the inducible nitric oxide synthase could improve the therapeutic outcome of patients suffering from either septic or hemorrhagic shock (104).

Nitric Oxide in Septic Shock

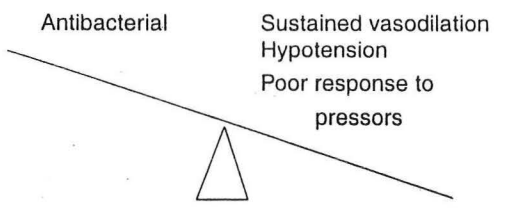
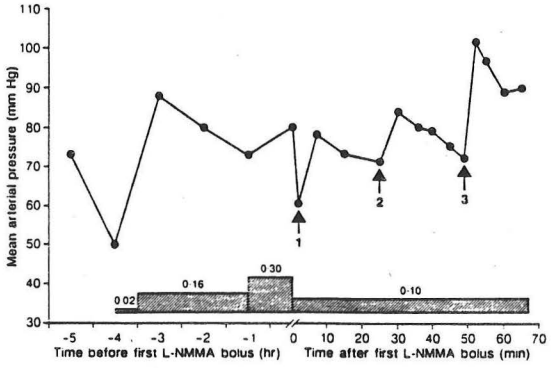


Figure 21

Several strategies have been proposed to inhibit nitric oxide production during shock: substituted L-arginine derivatives, dexamethasone to prevent induction of the inducible nitric oxide synthase, arginase to degrade circulating arginine, or a calmodulin inhibitor (33,105). Anecdotal experience in two patients with septic shock demonstrated that a nitric oxide synthase inhibitor (L-NMMA) reversed hypotension (108) [Figure 22]. In these two patients, conventional therapy had been unable to normalize blood pressure. In contrast, infusion of the nitric oxide synthase inhibitor increased the blood pressure and systemic vascular resistance, indicating that nitric oxide contributes to the pathogenesis of septic shock. L-NMMA infusion improves survival in a murine peritonitis model of sepsis that is not cured by antibiotics alone (109).

Effect of L-NMMA on Hypotension in a Patient with Septic Shock



Petros, et.al., Lancet, 1991

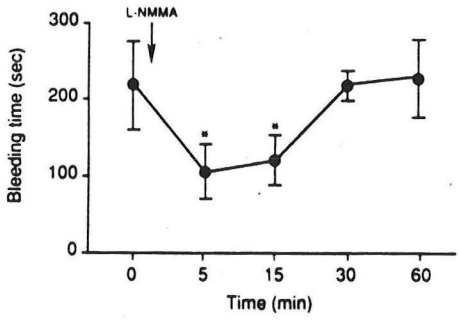
Figure 22

Nitric oxide synthase inhibitors would represent a welcome addition to our therapeutic armamentarium. However, before this therapy can be widely accepted, several issues need to be clarified. First, how much does nitric oxide contribute to the morbidity and mortality in sepsis? Second, how much inhibition of nitric oxide synthase is beneficial? Complete inhibition of nitric oxide synthesis would be counterproductive, perhaps causing to myocardial and tissue ischemia, platelet aggregation with microvascular thrombosis, breakdown of defence barriers in the skin and GI tract, and compromised antimicrobial defenses (106).

Uremic bleeding. Patients with uremia have prolonged bleeding time manifested as an increased incidence of GI bleeding. Although spontaneous bleeding is uncommon, the bleeding tendency is important to recognize in patients who must undergo surgery or renal biopsy. The incidence of bleeding has been reduced by early institution of dialysis therapy. The exact mediator is unknown; the list of candidates is very long (decreased platelet factor-3 or clotting factor-VIII, or increased prostacyclin, guanidinosuccinic acid, or phenolic acid). Current therapy for restoring bleeding time to normal includes DDAVP (0.3 μg/kg iv) or conjugated estrogens (0.6 mg/kg daily for 5 days) (81,110).

Because nitric oxide is known to inhibit platelet aggregation and adhesion, it was suggested that nitric oxide could be involved in the pathogenesis of the uremic bleeding. In rats with chronic renal failure, inhibition of nitric oxide synthase with L-NMMA completely normalizes bleeding time within 5 minutes; the effect lasts for approximately 30 minutes [Figure 23] (111). The inhibitor also increased platelet adhesion measured in vitro; in vivo and in vitro effects were completely reversed by arginine. Thus, it appears that nitric oxide is a mediator of the bleeding tendency of uremia. A follow up study showed that a beneficial effect of estrogen could be reversed by L-arginine (112). Dexamethasone also shortened the prolonged bleeding time of uremia. These results suggest that estrogen may decrease the inducible nitric oxide synthase. To date, these experiments have not been reported in humans, perhaps because of the potential for hypertension, and the side effects discussed in the section on septic shock.

L-NMMA Normalizes Bleeding Time In Uremic Rats



Remuzzi, et.al., J. Clin. Invest., 1990

Figure 23

CONCLUSION

This review has focused on some of the cardiovascular and platelet actions of nitric oxide. Nitric oxide has many other effects throughout the body. The widespread distribution of this signal transduction system complicates the use of general agonists (arginine) and inhibitors (L-NMMA). Drugs must be developed that selectively inhibit only one isoform of nitric oxide synthase, or perhaps a specific arginine transporter. Gene therapy may allow targeting of nitric oxide to specific organ beds (heart, kidneys, et.), where its short range of action may prove useful.

ACKNOWLEDGEMENTS

I appreciate the excellent secretarial support provided by Barbara Locke, graphics support of Dede Copeland, the library research by Laurie Hogarth, and the many helpful discussions with Drs. C. Richard Lyons, Philip Shaul, David Harrison, and L. David Hillis.

REFERENCES

1. Snyder, S.H. and D.S. Bredt. 1992. Biological roles of nitric oxide. *Sci. Am.* 266:68-77.

2. Lancaster, J.R. 1992. Nitric oxide in cells. *Am. Scientist* 80:248-259.

3. Bates, J.N., D.G. Harrison, P.R. Myers, and R.L. Minor. 1991. EDRF: nitrosylated compound or authentic nitric oxide. *Basic. Res. Cardiol.* 86 S2:17-26.

4. Stamler, J.S., D.I. Simon, J.A. Osborne, M.E. Mullins, O. Jaraki, T. Michel, D.J. Singel, and J. Loscalzo. 1992. S-Nitrosylation of proteins with nitric oxide: Synthesis and characterization of biologically active compounds. *Proc. Natl. Acad. Sci. USA* 89:444-448.

5. Stamler, J.S., O. Jaraki, J. Osborne, D.I. Simon, J. Keaney, J. Vita, D. Singel, C.R. Valeri, and J. Loscalzo. 1992. Nitric oxide circulates in mammalian plasma primarily as an *S*-nitroso adduct of serum albumin. *Proc. Natl. Acad. Sci. USA* 89:7674-7677.

6. Wilcox, C.S., W.J. Welch, F. Murad, S.S. Gross, G. Taylor, R. Levi, and H.H.H.W. Schmidt. 1992. Nitric oxide synthase in macula densa regulates glomerular capillary pressure. *Proc. Natl. Acad. Sci. USA* 89:11993-11997.

7. Moncada, S., R.M.J. Palmer, and E.A. Higgs. 1991. Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* 43:109-142.

8. Förstermann, U., H.H.H.W. Schmidt, J.S. Pollock, H. Sheng, J.A. Mitchell, T.D. Warner, M. Nakane, and F. Murad. 1991. Isoforms of nitric oxide synthase. Characterization and purification from different cell types. *Biochem. Pharmacol.* 42:1849-1857.

9. Brendt, D.S., P.M. Hwang, C.E. Glatt, C. Lowenstein, R.R. Reed, and S.H. Snyder. 1991. Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. *Nature* 351:714-718.

10. Lyons, C.R., G.J. Orloff, and J.M. Cunningham. 1992. Molecular cloning and functional expression of an inducible nitric oxide synthase from a murine macrophage cell line. *J. Biol. Chem.* 267:6370-6374.

11. Xie, Q., H.J. Cho, J. Calaycay, R.A. Mumford, K.M. Swiderek, T.D. Lee, A. Ding, T. Troso, and C. Nathan. 1992. Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. *Science* 256:225-228.

12. Lamas, S., P.A. Marsden, G.K. Li, P. Tempst, and T. Michel. 1992. Endothelial nitric oxide synthase: Molecular cloning and characterization of a distinct constitutive enzyme isoform. *Proc. Natl. Acad. Sci. USA* 89:6348-6352.

13. Sessa, W.C., J.K. Harrison, C.M. Barber, D. Zeng, M.E. Durieux, D.D. D'Angelo, K.R. Lynch, and M.J. Peach. 1992. Molecular cloning and expression of a cDNA encoding endothelial cell nitric oxide synthase. *J. Biol. Chem.* 267:15274-15276.

14. Marsden, P.A., K.T. Schappert, H.S. Chen, M. Flowers, C.L. Sundell, J.N. Wilcox, S. Lamas, and T. Michel. 1992. Molecular cloning and characterization of human endothelial nitric oxide synthase. *FEBS Lett.* 307:287-293.

15. Nishida, K., D.G. Harrison, J.P. Navas, A.A. Fisher, S.P. Dockery, M. Uematsu, R.M. Nerem, R.W. Alexander, and T.J. Murphy. 1992. Molecular cloning and characterization of the constitutive bovine aortic endothelial cell nitric oxide synthase. *J. Clin. Invest.* 90:2092-2096.

16. Ignarro, L.J. 1991. Signal transduction mechanisms involving nitric oxide. *Biochem. Pharmacol.* 41:485-490.

17. Granger, D.L. 1992. Macrophage production of nitric oxides in host defense against microorganisms. *Forum Immunol.* 39:570-572.

18. Corbett, J.A. 1992. Interluken 1 beta inducdes the formation of nitric oxide by beta-cells purified from rodent islets of Langerhans. Evidence for the beta-cell as a source and site of action of nitric oxide. *J. Clin. Invest.* 90:2384-2391.

19. Yuen, P.S.T., L.R. Potter, and D.L. Garbers. 1990. A new form of guanylyl cyclase is preferentially expressed in rat kidney. *Biochemistry* 29:10872-10878.

20. Schulz, S., P.S.T. Yuen, and D.L. Garbers. 1991. The expanding family of guanylyl cyclases. *TIPS* 12:116-120.

21. Yuen, P.S.T. and D.L. Garbers. 1992. Guanylyl cyclase-linked receptors. *Annu. Rev. Neurosci.* 15:193-225.

22. Ujiie, K., J.G. Drewett, P.S.T. Yuen, and R.A. Star. 1993. Differential expression of mRNA for guanylyl cyclase-linked endothelium-derived relaxing factor receptor subunits in rat kidney. *J. Clin. Invest.* 91:730-734.

23. Harteneck, C., B. Wedel, D. Koesling, J. Malkewitz, E. Bohme, and G. Schultz. 1991. Molecular cloning and expression of a new α-subunit of soluble guanylyl cyclase: Interchangeability of the α-subunits of the enzyme. *FEBS Lett.* 292:217-222.

24. Nakazono, K., N. Watanabe, K. Matsuno, J. Sasaki, T. Sato, and M. Inoue. 1991. Does superoxide underlie the pathogenesis of hypertension. *Proc. Natl. Acad. Sci. USA* 88:10045-10048.

25. Vallance, P., A. Leone, A. Calver, J. Collier, and S. Moncada. 1992. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 339:572-575.

26. Vallance, P., A. Leone, A. Calver, J. Collier, and S. Moncada. 1992. Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. *J. Cardiovasc. Pharmacol.* 20 Suppl. 12:S60-S62.

27. Victor, R. Uremic autonomic neuropathy: The ups and downs of blood pressure in patients with chronic renal failure. *Medical Grand Rounds* October 22, 1992:

28. Lüscher, T.F., T.M. Masaki, G.M. Rubanyi, P.M. Vanhoutte, and J.R. Vane. 1992. 2nd International Symposium on Endothelium-Derived Vasoactive Factors, proceedings of a meeting held in Basel, Switzerland, April 21-25, 1992. Introduction. *J. Cardiovasc. Pharmacol.* 20 Suppl. 12:vii-viii.

29. Knowles, R.G. and S. Moncada. 1992. Nitric oxide as a signal in blood vessels. *Trends Biochem. Sci.* 17:399-402.

30. Furchgott, R.F. and J.V. Zawadzki. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288:373-376.

31. Danziger, R.S., W.B. Campbell, and R.A. Star. 1992. Molecular characterization of the endothelial EDRF receptor and calcium signalling by cGMP in cultured bovine coronary endothelial cells. *Am. Heart Assoc.* (Abstract)

32. Palmer, R.M.J., L. Bridge, N.A. Foxwell, and S. Moncada. 1992. The role of nitric oxide in endothelial cell damage and its inhibition by glucocorticoids. *Br. J. Pharmacol.* 105:11-12.

33. Radomski, M.W., R.M.J. Palmer, and S. Moncada. 1990. Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proc. Natl. Acad. Sci. USA* 87:10043-10047.

34. Mitchell, J.A., M. Hecker, E.E. Anggard, and J.R. Vane. 1990. Cultured endothelial cells maintain their L-arginine level despite the continuous release of EDRF. *Eur. J. Pharmacol.* 182:573-576.

35. Hecker, M., W.C. Sessa, H.J. Harris, E.E. Änggård, and J.R. Vane. 1990. The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived relaxing factor: Cultured endothelial cells recycle L-citrulline to L-arginine. *Proc. Natl. Acad. Sci. USA* 87:8612-8616.

36. Gardiner, S.M., A.M. Compton, T. Bennett, R.M.J. Palmer, and S. Moncada. 1990. Control of regional blood flow by endothelium-derived nitric oxide. *Hypertension* 15:486-492.

37. Dinh-Xuan, A.T., T.W. Higenbottam, C.A. Clelland, J. Pepke-Zaba, G. Cremona, Y. Butt, S.R. Large, F.C. Wells, and J. Wallwork. 1991. Impairment of endothelium-dependent pulmonary-artery relaxation in chronic obstructive lung disease. *N. Engl. J. Med.* 324:1539-1547.

38. Rossaint, R., K.J. Falke, F. Lopez, K. Slama, U. Pison, and W.M. Zapol. 1993. Inhaled nitric oxide for the adult respiratory distress syndrome. *N. Engl. J. Med.* 328:399-405.

39. Bone, R.C. 1993. A new therapy for the adult respiratory distress syndrome. *N. Engl. J. Med.* 328:431-432.

40. Stark, M.E. and J.H. Szurszewski. 1992. Role of nitric oxide in gastrointestinal and hepatic function and disease. *Gastroenterology* 103:1928-1949.

41. Rees, D.D., R.M.J. Palmer, and S. Moncada. 1989. Role of endothelium-derived nitric

oxide in the regulation of blood pressure. *Proc. Natl. Acad. Sci. USA* 86:3375-3378.

42. Hishikawa, K., T. Nakaki, M. Tsuda, H. Esumi, H. Ohshima, H. Suzuki, T. Saruta, and R. Kato. 1992. Effect of systemic l-arginine administration on hemodynamics and nitric oxide release in man. *Jpn. Heart J.* 33:41-48.

43. Nakaki, T., K. Hishikawa, H. Suzuki, T. Saruta, and R. Kato. 1990. L-arginine-induced hypotension. *Lancet* 336:696.

44. Togashi, H., I. Sakuma, M. Yoshioka, T. Kobayashi, H. Yasuda, A. Kitabatake, H. Saito, S.S. Gross, and R. Levi. 1990. A central nervous system action of nitric oxide in blood pressure regulation. *J. Pharmacol. Exp. Ther.* 262:343-347.

45. Sanders, P.W. 1993. Role of nitric oxide in regulation of blood pressure. *J. Nephrol.* 5:23-30.

46. Harrison, D.G., C.B. Treasure, A. Mügge, K.C. Dellsperger, and K.G. Lamping. 1991. Hypertension and the coronary circulation: With special attention to endothelial regulation. *Am. J. Hypertens.* 4 Suppl.:454S-459S.

47. Baylis, C., B. Mitruka, and A. Deng. 1992. Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. *J. Clin. Invest.* 90:278-281.

48. Chen, P.Y. and P.W. Sanders. 1991. L-Arginine abrogates salt-sensitive hypertension in Dahl/Rapp rats. *J. Clin. Invest.* 88:1559-1567.

49. Chen, P.Y., P.L. St.John, K.A. Kirk, D.R. Abrahamson, and P.W. Sanders. 1993. Hypertensive nephrosclerosis in Dahl/Rapp rat. *Lab. Invest.* (In Press)

50. Martasek, P., M.L. Schwartzman, A.I. Goodman, K.B. Solangi, R.D. Levere, and N.G. Abraham. 1991. Hemin and L-arginine regulation of blood pressure in spontaneous hypertensive rats. *J. Am. Soc. Neph.* 2:1078-1084.

51. Shultz, P.J. and J.P. Tolins. 1993. Adaptation to increased dietary salt intake in the rat. *J. Clin. Invest.* 91:642-650.

52. Di Nicola, L., R.C. Blantz, and F.B. Gabbai. 1992. Nitric oxide and angiotensin II. Glomerular and tubular interaction in the rat. *J. Clin. Invest.* 89:1248-1256.

53. Stoos, B.A., O.A. Carretero, R.D. Farhy, G. Scicli, and J.L. Garvin. 1992. Endothelium-derived relaxing factor inhibits transport and increases cGMP content in cultured mouse cortical collecting duct cells. *J. Clin. Invest.* 89:761-765.

54. Calver, A., J. Collier, S. Moncada, and P. Vallance. 1992. Effect of local intra-arterial N^G-monomethyl-L-arginine in patients with hypertension: The nitric oxide dilator mechanism appears abnormal. *J. Hypertens.* 10:1025-1031.

55. Panza, J.A., A.A. Quyyumi, Jr Brush,J.E., and S.E. Epstein. 1990. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N. Engl. J. Med.* 323:22-27.

56. Linder, L., W. Kiowski, F.R. Buhler, and T.F. Luscher. 1990. Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation in vivo. *Circulation* 81:1762-1767.

57. Vallance, P., J. Collier, and S. Moncada. 1989. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* 2:997-1000.

58. Hishikawa, K., T. Nakaki, H. Suzuki, R. Kato, and T. Saruta. 1992. L-Arginine as an antihypertensive agent. *J. Cardiovasc. Pharm.* 20(S12):S196-S197.

59. Hishikawa, K., T. Nakaki, H. Suzuki, T. Saruta, and R. Kato. 1991. L-arginine-induced hypotension. *Lancet* 337:683-684.

60. Bowlby, H.A. and S.I. Elanjian. 1992. Necrosis caused by extravasation of arginine hydrochloride. *Ann. Pharm.* 26:263-264.

61. Taddei, S., A. Virdis, P. Mattei, F. Arzilli, and A. Salvetti. 1992. Endothelium-dependent forearm vasodilation is reduced in normotensive subjects with familial history of hypertension. *J. Cardiovasc. Pharm.* 20(S12):S193-S195.

62. Amezcua, J.L., R.M. Palmer, B.M. deSousa, and S. Moncada. 1989. Nitric oxide synthesized from L-arginine regulates vascular tone in the coronary circulation of the rabbit. *Br. J. Pharmacol.* 97:1119-1124.

63. Chu, A., D.E. Chamers, C.C. Lin, W.D. Kuehl, R.M. Palmer, S. Moncada, and F.R. Cobb. 1991. Effects of inhibition of nitric oxide formation on basal vasomotion and endothelium-dependent responses of the coronary arteries in awake dogs. *J. Clin. Invest.* 87:1964-1968.

64. Mugge, A., U. Forestermann, and P.R. Lichtlen. 1991. Platelets, endothelium-dependent responses and atherosclerosis. *Ann. Med.* . 23:545-550.

65. Harrison, D.G., M.A. Kurz, J.E. Quillen, F.W. Sellke, and A. Mügge. 1992. Normal and pathophysiologic considerations of endothelial regulation of vascular tone and their relevance to nitrate therapy. *Am. J. Cardiol.* 70:11B-17B.

66. Maseri, A. 1991. Coronary vasoconstriction: visible and invisible. *N. Engl. J. Med.* 325:1579-1580.

67. Ludmer, P.L., A.P. Selwyn, T.L. Shook, R.R. Wayne, G.H. Mudge, W. Alexander, and P. Ganz. 1986. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N. Engl. J. Med.* 315:1046-1051.

68. Bassenge, E. 1992. Clinical relevance of endothelium-derived relaxing factor (EDRF). *Br. J. Clin. Pharmacol.* 34 Suppl. 1:37S-42S.

69. Zieher, A.M., H. Drexler, H. Wollschlager, and H. Just. 1991. Endothelial dysfunction of the coronary microvasculature is associated with impaired. *Circulation* 84:1984-1992.

70. Zieher, A.M., H. Drexler, H. Wollschlager, and H. Just. 1991. Modulation of coronary vasomotor tone in humans. *Circulation* 83:391-401.

71. Dubois-Rande, J-L., R. Zelinsky, P.E. Chabrier, A. Castaigne, H. Geschwind, and S. Adnot. 1992. L-arginine improves endothelim-dependent relaxation of conductance and resistance coronary. *J. Cardiovasc. Pharm.* 20(S12):S211-S213.

72. Simon, B.C., L.D. Cunningham, and R.A. Cohen. 1990. Oxidized low density lipoproteins cause contraction and inhibit endothelium-dependent relaxation in the pig coronary artery. *J. Clin. Invest.* 86:75-79.

73. Bucala, R., K.J. Tradey, and A. Cerami. 1991. Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilation in experimental diabetes. *J. Clin. Invest.* 87:432-438.

74. Drexler, H., A.M. Zeiher, K. Meinzer, and H. Just. 1991. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolaemic patients by l-arginine. *Lancet* 338:1546-1550.

75. Radomski, M.W., R.M.J. Palmer, and S. Moncada. 1991. Modulation of platelet aggregation by an L-arginine-nitric oxide pathway. *TIPS* 12:87-88.

76. Golino, P., F. Piscione, J.T. Willerson, M. Cappelli-Bigazzi, A. Focaccio, B. Villari, C. Indolfi, E. Russolillo, M. Condorelli, and M. Chiariello. 1991. Divergent effects of serotonin on coronary artery dimensions and blood flow in patients with coronary atherosclerosis and control patients. *N. Engl. J. Med.* 324:641-648.

77. McFadden, E.P., J.G. Clarke, G.J. Davies, J.C. Kaski, A.W. Haider, and A. Maseri. 1991. Effect of intracoronary serotonin on coronary vessels in patients with stable angina and patients with varient angina. *N. Engl. J. Med.* 324:648-654.

78. Chester, A.H., G.S. O'Neil, S. Moncada, S. Tadjkarimi, and M.H. Yacoub. 1990. Low basal and stimulated release of nitric oxide in atherosclerotic epicardial coronary arteries. *Lancet* 336:897-900.

79. Egashira, K., T. Inou, Y. Hirooka, A. Yamada, Y. Maruoka, H. Kai, S. Sugimachi, S. Suzuki, and A. Takeshita. 1993. Impaired coronary blood flow response to acetylcholine in patients with coronary risk factors and proximal atherosclerotic lesions. *J. Clin. Invest.* 91:39-37.

80. Creager, M.A., J.P. Cooke, M.E. Mendelsohn, S.J. Gallagher, S.M. Coleman, J. Loscalzo, and V.J. Dzau. 1990. Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. *J. Clin. Invest.* 86:228-234.

81. Calver, A., J. Collier, and P. Vallance. 1992. Inhibition and stimulation of nitric oxide synthesis in the human forearm arterial bed of patients with insulin-dependent diabetes. *J. Clin. Invest.* 90:2548-2554.

82. Werns, S.W., J.A. Walton, H.H. Hsia, E.G. Nabel, M.L. Sanz, and B. Pitt. 1989. Evidence of endothelial dysfunction in agiographically normal coronary arteries of patients with coronary artery disease. *Circulation* 79:287-291.

83. Newman, C.M., A. Maseri, D.R. Hackett, H.M. El-Tamimi, and G.J. Davies. 1990. Response of angiographically normal and atherosclerotic left anterior descending coronary arteries to acetylcholine. *Am. J. Cardiol.* 66:1070-1076.

84. Motz, W., M. Vogt, O. Rabenau, S. Scheler, A. Luckhoff, and B.E. Strauer. 1991. Evidence of endothelial dysfunction in coronary resistance vessels in patients with angina pectoris and normal coronary angiogram. *Am. J. Cardiol.* 68:996-1003.

85. Maseri, A., F. Crea, J.C. Kaski, and T. Crane. 1991. Mechanisms of angina pectoris in syndrome X. *J. Am. Coll. Cardiol.* 17:499-506.

86. Freiman, P.C., G.G. Michell, D.D. Heistad, M.L. Armstrong, and D.G. Harrison. 1986. Atherosclerosis impairs endothelium-dependent vascular relaxation to acetylcholine and thrombin in primates. *Circ. Res.* 58:783-789.

87. Harrison, D.G., M.L. Armstrong, P.C. Freiman, and D.D. Heistad. 1987. Restoration of endothelium-dependent relaxation by dietary treatment of atherosclerosis. *J. Clin. Invest.* 80:1808-1811.

88. Atkins, J.M. Nitroglycerin explosions, tolerance, and myths. *Medical Grand Rounds* Aug 10, 1989:

89. Van de Voorde, J. 1991. Mechanisms involved in the development of tolerance to nitrovasodilators. *J. Cardiovasc. Pharmacol.* 17 Suppl. 3:S304-S308.

90. May, D.C., J.J. Popma, W.H. Black, S. Schaefer, H.R. Lee, B.D. Levine, and L.D. Hillis. 1987. In vivo induction and reversal of nitroglycerin tolerance in human coronary arteries. *N. Engl. J. Med.* 317:805-809.

91. Hibbs, J.B.,Jr., C. Westerfelder, R. Taintor, Z. Vavrin, C. Kablitz, R.L. Baranowski, J.H. Ward, R.L. Menlove, M.P. McMurry, J.P. Kushner, and W.E. Samlowski. 1992. Evidence for cytokine-inducible nitric oxide synthesis from L-arginine in patients receiving interleukin-2 therapy. *J. Clin. Invest.* 89:867-877.

92. Dawson, V.L., T.M. Dawson, E.D. Longon, D.S. Bredt, and S.H. Snyder. 1991. Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proc. Natl. Acad. Sci. USA* 88:6368-6371.

93. Nguyen, T., D. Brunson, C.L. Crespi, B.W. Penman, J.S. Wishnok, and S.R. Tannenbaum. 1992. DNA damage and mutation in human cells exposed to nitric oxide in vitro. *Proc. Natl. Acad. Sci. USA* 89:3030-3034.

94. Beckman, J.S., T.W. Beckman, J. Chen, P.A. Marshall, and B.A. Freeman. 1990. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA* 87:1620-1624.

95. Stadler, J., T.R. Billiar, R.D. Curran, D.J. Stuehr, J.B. Ochoa, and R.L. Simmons. 1991. Effect of exogenous and endogenous nitric oxide on mitochondrial respiration of rat hepatocytes. *Am. J. Physiol.* 260:C910-C916.

96. Stuehr, D.J. and C.F. Nathan. 1989. Nitric Oxide. A macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. *J. Exp. Med.* 169:1543-1555.

97. Hibbs Jr, J.B., R.R. Taintor, and Z. Vavrin. 1987. Macrophage cytotoxicity: role for L-arginine deiminase and imino nitrogen oxidation to nitrite. *Science* 235:473-476.

98. Drapier, J-C. and J.B. Hibbs Jr. 1986. Murine cytotoxic activated macrophages inhibit aconitase in turmor cells. *J. Clin. Invest.* 78:790-797.

99. Granger, D.L. and A.L. Lehninger. 1982. Sites of inhibition of mitochondrial electron transport in macrophage-injured neoplastic cells. *J. Cell Biol.* 95:527-535.

100. Alspaugh, J.A. and D.L. Granger. 1991. Inhibtion of cryptococcus neoformans replication by nitrogen oxides supports the role of these molecules as effectors of macrophage-mediated cytostasis. *Infect. Immun.* 59:2291-2296.

101. Liew, F.Y., S. Millot, C. Parkinson, R.M.J. Palmer, and S. Moncada. 1990. Macrophage killing of leishmania parasites in vivo is mediated by nitric oxide from L-arginine. *J.*

Immunology 144:4794.

102. Knowles, R.G., M. Salter, S.L. Brooks, and S. Moncada. 1990. Anti-inflammatory glucocorticoids inhibit the induction by endotoxin of nitric oxide synthase in the lung, liver and aorta of the rat. *Biochem. Biophys. Res. Commun.* 172:1042-1048.

103. Lamas, S., T. Michel, B.M. Brenner, and P.A. Marsden. 1991. Nitric oxide synthesis in endothelial cells: Evidence for a pathway inducible by TNF-*α*. *Am. J. Physiol. Cell Physiol.* 261:C634-C641.

104. Thiemermann, C., C. Szabó, J.A. Mitchell, and J.R. Vane. 1993. Vascular hyporeactivity to vasoconstrictor agents and hemodynamic decompensation in hemorrhagic shock is mediated by nitric oxide. *Proc. Natl. Acad. Sci. USA* 90:267-271.

105. Rees, D.D., S. Cellek, R.M.J. Palmer, and S. Moncada. 1990. Dexamethasone prevents the induction by endotoxin of a nitric oxide synthase and the associated effects on vascular tone: An insight into endotoxin shock. *Biochem. Biophys. Res. Commun.* 173:541-547.

106. Hotchkiss, R.S., I.E. Karl, J.L. Parker, and H.R. Adams. 1992. Inhibition of NO synthesis in septic shock. *Lancet* 339:434-435.

107. Schmidt, H.H.H.W., T.D. Warner, and F. Murad. 1992. Double-edged role of endogenous nitric oxide. *Lancet* 339:986.

108. Petros, A., D. Bennett, and P. Vallance. 1991. Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock. *Lancet* 338:1557-1558.

109. Teale, D.M. and A.M. Atkinson. 1992. Inhibition of nitric oxide synthesis improves survival in a murine peritonitis model of sepsis that is not cured by antibiotics alone. *J. Antimicrob. Chemother.* 30:839-842.

110. Mannucci, P.M., G. Remuzzi, and F. Pusineri. 1983. Desamino-8-o-arginine vasopressin shortens the bleeding time in uremia. *N. Engl. J. Med.* 308:8.

111. Remuzzi, G., N. Perico, C. Zoja, D. Corna, D. Macconi, and G. Vigano. 1990. Role of endothelim-derived nitric oxide in the bleeding tendency of uremia. *J. Clin. Invest.* 86:1768-1771.

112. Zoja, C., M. Noris, D. Corna, G. Viganò, N. Perico, G. De Gaetano, and G. Remuzzi. 1991. L-arginine, the precursor of nitric oxide, abolishes the effect of estrogens on bleeding time in experimental uremia. *Lab. Invest.* 65:479-483.