Physiological and Clinical Significance of Nitric Oxide

Toshio Nakaki

Department of Pharmacology, School of Medicine, Keio University, Tokyo, Japan

(Received for publication on October 8, 1993)

Abstract. Nitric oxide, or NO', is a gas under atmospheric conditions. It is noxious because of its free-radical structure. It is biosynthesized from the amino acid L-arginine. The responsible enzymes are widely distributed in the human body. It has been shown that this simple molecule, NO', plays important roles in mammalian physiology. It is one of the factors regulating vascular tone and blood pressure, inhibition of platelet aggregation, neurotransmission in the peripheral and central nervous systems and macrophage function. Evidence for the pathophysiological significance of NO' is now accumulating. (Keio J Med 43 (1): 15-26, March 1994)

Key words: endothelium-derived relaxing factor, nitrogen monoxide, nitrosonium

Introduction

In 1980, a pharmacologist and profound scholar, Robert Furchgott, who had been working solely with a classical organ bath assay for thirty years and never used "modern" techniques such as receptor binding assay or molecular biology, made an epoch-making observation, which attracted the attention of numerous investigators to the endothelium. He discovered that acetylcholine relaxes rabbit aortic smooth muscles only when the endothelium is intact.\(^1\) This discovery was not achieved by serendipity.

The story began in 1914 when Sir Henry Dale first observed that intravenous injection of acetylcholine increased blood flow in the arteries of the rabbit ear and dilated vessels in vivo.\(^2\) Investigators attempted to confirm acetylcholine's action in vitro, but without success. The mystery for researchers including Furchgott himself was that the isolated rabbit artery contracted, but did not relax in response to acetylcholine in vitro. In 1962, a paper appeared which described blood vessel relaxation with acetylcholine.\(^3\) The author used a new method of preparing rabbit aorta, using a ring preparation in place of the helical strip which Furchgott had developed and gained expertise with. Furchgott repeated these experiments and confirmed that acetylcholine relaxes the ring preparation, but not the helical strip preparation of rabbit aorta. Furchgott identified the source of this apparent contradiction. It turned out that the endothelium had been inadvertently injured during preparation of the helical strip. He demonstrated that acetylcholine-induced relaxation is endothelium-dependent and that even in the ring preparation acetylcholine contracted the rabbit aorta if the endothelium had been removed.\(^1\) He reasoned that there must be an unidentified substance, which was released from the endothelium, capable of relaxing the smooth muscles of blood vessels. This observation and speculation opened a new era of endothelium research.

Now, in 1994 we know that EDRF (endothelium-derived relaxing factor) is nitric oxide; NO is enzymatically formed from L-arginine; NO synthase (NOS) has a unique structure in that the enzyme is active with cofactors including FMN, FAD, calmodulin, heme and tetrahydrobiopterin; NOS is broadly divided to constitutive NOS (cNOS) and inducible NOS (iNOS) forms. The cNOS form is found in endothelial cells, platelets and megakaryoblastic cells, bronchial epithelial cells, the cerebellum, parasympathetic nerves and the macula densa. iNOS is widely distributed in macrophages, vascular endothelium and smooth muscles, cardiac myocytes, kidney mesangial cells, chondrocytes, glial cells (astrocytes and microglial cells), and Kupffer cells; NO activates guanylate cyclase and stimulates the production of cyclic GMP; the biological activity of NO, however, may not be attributable solely to an increase in cellular cyclic GMP. NO is not merely an object of research curiosity. Nitroglycerin has long been used for the treatment of

---

*Reprint requests to: Dr Toshio Nakaki, Department of Pharmacology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan*
angina pectoris and its active substance is NO. Accumulating lines of evidence show that NO is profoundly involved in physiological and pathophysiological processes in humans. This brief review describes physiological and pathophysiological aspects of nitric oxide. Readers are recommended to see recent reviews on NO.4-9 This review was completed in the early part of September, 1993.

The nomenclature of NO should be briefly elaborated upon.10 The designation nitric oxide should be restricted to the reduced NO form of the molecule, while the parent NO compound is termed nitrogen monoxide. The oxidized form, NO+, is the nitrosonium ion. NO−, in which one electron has been added to the nitric oxide form, is the nitrosyl anion. All three nitrogen monoxide forms are interchangeable, which increases the complexity of the biochemical mechanisms of this molecule. The species which can activate guanylate cyclase is at present considered to be NO−. Certain drugs, such as nitroglycerin and sodium nitroprusside, are of NO+ character and thus must be reduced intracellulary to NO− in order to activate guanylate cyclase. In this review, the term NO means NO− unless otherwise stated, since it is difficult to define which of the molecular species is being discussed in each of the original articles to be cited herein. Nitric oxide is different from the anesthetic gas, nitrous oxide, or N2O.

Although several lines of convincing evidence have shown that EDRF is NO−, there is still skepticism as to whether EDRF may be some other molecule very similar to NO− such as an S-nitrosylated compound. In this review, however, EDRF and NO− are treated as being synonymous.

Cardiovascular Systems

Vascular tone and blood pressure

The basal release of NO derived from L-arginine plays an important role in regulating blood flow and pressure. Evidence for this comes from the observation that, in rabbits, intravenous administration of a specific inhibitor of NO, NG-monomethyl-L-arginine (L-NMMA) induced an increase in blood pressure that was reversible with L-arginine, but not D-arginine.11 The importance of NO in regulating basal tone in resistance vessels was demonstrated using an NOS inhibitor, L-NMMA, in the isolated coronary circulation.12 The importance of NO in regulating blood flow has also been demonstrated in humans. Studies in which L-NMMA was infused into the human brachial artery or hand dorsal veins demonstrated that vasodilation could be induced by either acetylcholine or bradykinin. Furthermore, whereas in the brachial artery L-NMMA induced direct vasoconstriction, it had no such effect on the hand veins. This observation suggests that on the arterial, but not the venous, side of the circulation there is a continuous release of NO which maintains dilator tone in humans.13

In the microcirculation, NO plays a role in regulating the vascular tone of arterioles and venules, although there are some variations among tissue preparations.14,15 The physiological stimuli for generation of NO are not yet fully understood, but pulsatile flow and shear stress appear to be two of the main determinants.16 Interestingly, it was reported that in resistance arteries of the intact rabbit ear, endothelium-dependent vasodilatation in response to acetylcholine was enhanced at increased flow rates.17 Flow-dependent coronary artery dilation has also been demonstrated in humans.18 The three best understood components of hemodynamic force are shear stress, wall tension, and transmural pressure. However, it is difficult to evaluate the effects of transmural pressure alone on vessels in situ. According to the law of Laplace, transmural pressure in a distensible hollow object causes wall tension, which may stretch endothelial cells. In an experimental system where shear stress and stretch can be excluded as much as possible by exposing cells cultured on flat, solid flasks to elevated pressure, we showed that pure pressure inhibits NO formation/release from human umbilical vein endothelial cells.19 A pressure of 80 mmHg caused maximal inhibition. Since the mean arterial pressure in healthy humans is in the 80 to 100 mmHg range, these results suggest that NO release may be inhibited, to some extent, under physiological conditions.

Platelets

NO derived from endothelial cells inhibits the adhesion and aggregation of platelets.5,20 NO-mediated inhibition of adhesion involves cyclic GMP, while that of aggregation involves cyclic AMP as well as cyclic GMP.21 Prostacyclin, which is a cyclic AMP-dependent agent, and NO acted synergistically to inhibit aggregation.22 Sodium nitroprusside, an NO donor, prolongs fibrinolysis, possibly by preventing the release from platelets of an inhibitor of tissue plasminogen activator.23 It has been shown that platelets also generate NO and that the autocrine L-arginine-NO pathways acts as a negative feedback mechanism to regulate platelet aggregation.24 eNOS in the platelet is similar to that of endothelium with respect to cofactor requirements and substrate specificity. Thus, aggregation induced by collagen was accompanied by an increase in intraplatelet levels of cyclic GMP. L-NMMA enhanced the aggregation. An intriguing observation is that L-arginine does not increase the basal levels of cyclic GMP in unstimulated platelets, doing so only when stimulated with collagen, showing that this NO synthase can utilize exogenous L-arginine as a substrate after it has been
activated. This suggests that a decrease in the plasma concentration of L-arginine leads to impaired negative feedback of aggregation and thereby enhanced aggregation.

**Leukocytes**

Endogenous NO derived from endothelial cells tonically inhibits the adhesion and aggregation of leukocytes to endothelium. Inhibitors of NOS lead to increased expression of CD11/CD18 adhesion molecules on neutrophils, the enhanced CD11/CD18-dependent attachment of the cells to the vessel wall, and their increased emigration into extra vascular tissue.

LPS-induced hepatotoxicity, intestinal tissue damage, and vascular permeability were exacerbated by administration of an NOS inhibitor and ameliorated by administration of S-nitroso-N-acetyl-penicillamine, an NO donor. In contrast to these findings, however, the same NOS inhibitor administered to the same species blocked immune complex-induced, neutrophil-dependent vascular injury in the lung.

Although endogenous NO appears to exert both pro- and antiinflammatory effects, this might be attributable to redox variables affecting the function of NO. The importance of the redox environment will be discussed below, in the section on neuronal death.

**Cardiac muscle**

Carbachol, a muscarinic agonist, caused 91% inhibition of the spontaneous beating rate of cultured neonatal rat cardiac myocytes. L-NMMA blocked the negative chronotropic effect of carbachol. This inhibitor significantly increased the inotropic effect of the β-adrenergic agonist isoproterenol on electrically stimulated adult rat ventricular myocytes. These observations suggest that the physiologic responses of isolated neonatal and adult ventricular myocytes to both muscarinic cholinergic and β-adrenergic stimulation are mediated, in part, by NO.

**Renin release**

Endogenous NO has been shown to inhibit the release of renin. In fact, the presence of NOS in the rat macula densa demonstrated by immunoreactivity. Tubuloglomerular feedback, which is mediated by the macula densa, appears to be regulated in part by NO. The ability of NO to regulate arteriolar diameter in the kidney may be a key factor in renin release.

Inflammatory stimuli such as cytokines and LPS induce iNOS in mesangial cells. This mechanism may play a role in nephritis.

**Hypertension**

It may be reasonable to speculate as to whether, in hypertensive patients, the vasorelaxing substance NO is reduced in amount. It has been known that basal forearm blood flow is similar in hypertensive patients and controls. Blood flow and vascular resistance responses to acetylcholine were significantly reduced in hypertensive patients, whereas the response to sodium nitroprusside, an NO donor, was unchanged or enhanced in such patients. L-Arginine did not significantly change basal blood flow or vascular resistance in either group. However, the infusion of L-arginine significantly augmented the vasodilator response to acetylcholine in normal control subjects. In contrast, in hypertensive patients, the infusion of L-arginine did not alter the response to acetylcholine. These results suggest that agonist-stimulated L-arginine availability is impaired in hypertensive subjects. However, we have shown that the administration of L-arginine induced a significant reduction in blood pressure in hypertensive, to an extent much greater than that of healthy normal controls. The blood pressure reduction was accompanied by increases in the plasma concentrations of cyclic GMP and L-citrulline, the latter being a byproduct of NO formation from L-arginine. Furthermore, urinary output of NO2-/NO3- was significantly increased. Provided the effect of L-arginine involves endogenous NO release, these data suggest that even in hypertensive patients the L-arginine-NO pathway remains operative when sufficient quantities of the substrate L-arginine are available. The precise mechanisms of L-arginine-induced hypotension await further investigation.

It has been reported that endothelial cells (cNOS) and smooth muscle cells (iNOS) from genetically hypertensive rats release less nitric oxide than cells from normotensive animals. This result does not necessarily indicate that defective NOS genes are responsible for the hypertension in this model. Alternative explanations include a biochemical defect, such as biosynthesis of a cofactor commonly required for activation of both forms of NOS. Although numerous studies have been published on animal models of hypertension, these studies are not entirely consistent. In fact, they are not strictly comparable, because different vascular preparations from different species stimulated with different agonists were used. There is a need for a more systematic study of the dysfunction, if any, underlying hypertension.

In patients with chronic renal failure, the plasma concentration of the endogenous NOS inhibitor, NG, NG-dimethyl-arginine, is increased to a level which is sufficient for inhibiting NOS. Therefore, this endogenous inhibitor is a potential factor in the hypertension associated with chronic renal failure.
Impaired NO-related, endothelium-dependent vasorelaxation has been observed in the pulmonary arterial hypertension of chronic obstructive lung disease.\textsuperscript{41}

Therapeutic trials with inhaled NO have been conducted for adult respiratory distress syndrome,\textsuperscript{42} congenital heart disease\textsuperscript{43} and pulmonary hypertension.\textsuperscript{44} For example, an inhaled dose as small as 40 ppm NO decreased pulmonary arterial pressure with no associated hemodynamic change. Inhaled NO induces selective pulmonary vasodilatation while improving gas exchange.\textsuperscript{45}

**Septic shock**

Septic shock is associated with bacterial lipopolysaccharide and release of numerous cytokines. The NOS inhibitor, L-NMMA, reversed the hypotension caused by bacterial lipopolysaccharide and tumor necrosis factor. This inhibitor was administered to patients in septic shock and reversed their hypotension.\textsuperscript{46} Therefore, NO of vascular origin may play a role in the hypotension associated with the administration of LPS or cytokines, and perhaps in septic shock.

It was reported that cytokines induce iNOS in both endothelium,\textsuperscript{47} and vascular smooth muscle cells. It was stated that tumor necrosis factor-\(\alpha\) by itself induces iNOS in cultured smooth muscle cells.\textsuperscript{48} We found that bacterial lipopolysaccharide, inadvertently contaminated with buffers, induced iNOS in the vascular wall.\textsuperscript{49} Therefore, we reexamined the ability of tumor necrosis factor-\(\alpha\) to induce iNOS and found that in the lipopolysaccharide-controlled condition the cytokine alone failed to induce the enzyme, while the cytokine in combination with LPS or interleukin 1\(\beta\) did induce iNOS in vascular smooth muscle cells.\textsuperscript{50} It is noteworthy that the combination of cytokines is capable of inducing iNOS in an LPS-independent manner. This may be relevant to the pathophysiology of hemorrhagic shock, as will be discussed below. The mRNA of iNOS is expressed by the combination of tumor necrosis factor and interleukin 1 in vascular smooth muscle cells,\textsuperscript{51} whereas in vascular endothelial cells the combination of tumor necrosis factor and interferon \(\gamma\) induces marked iNOS mRNA production.\textsuperscript{52} The amount of NO produced under these conditions appears to be much greater than that produced by agonist-triggered endothelial cells.

Paradoxically, survival after lipopolysaccharide challenge was significantly enhanced by previous treatment with a microdose of lipopolysaccharide.\textsuperscript{53} This phenomenon has been interpreted as down regulation of the NO-guanylate system by prior administration of the lipopolysaccharide.

**Hemorrhagic shock**

Hemorrhagic shock causes a time-dependent reduction in the pressor responses to norepinephrine. This hyporeactivity is mediated by an enhanced release of NO by cNOS, for it is reversible with NG-nitro-L-arginine methyl ester, an inhibitor of both cNOS and iNOS, but it cannot be prevented by dexamethasone, an inhibitor of NOS induction. Vascular decompensation following prolonged periods of hemorrhagic shock is characterized by a failure, control animals, to maintain arterial blood pressures despite infusion of blood. This progressive decrease in blood pressure is mediated by enhanced formation of NO by the iNOS, as evidenced by its prevention by the NOS inhibitor. These observations suggest that excessive NO formation induces vascular hyporeactivity and decompensation in hemorrhagic shock.\textsuperscript{54} It is known that concentrations of cytokines are increased in plasma during hemorrhagic shock.\textsuperscript{55}

**Atherosclerosis**

Atherosclerotic coronary arteries display diminished vasodilatation in response to acetylcholine, as compared with normal coronary arteries.\textsuperscript{56,57}

A decrease in endothelium-dependent relaxation was first reported by Verbeuren et al (1986).\textsuperscript{58} Many convincing studies have since been published, showing a reduction in the release of EDRF from the vascular endothelium in the atherosclerotic state.\textsuperscript{59,60} This suggests impaired endothelial function in atherosclerosis, although it is not clear whether these abnormal responses are primary or secondary to the disease.

Diminished NO production may accelerate coronary artery disease by promoting interactions between platelets and the vessel wall through loss of NO-mediated platelet inhibition.\textsuperscript{20} Furthermore, NO may play an important role in maintaining the normal quiescent state of vascular smooth muscle, since NO-producing vasodilators\textsuperscript{61} and NO\textsuperscript{62} inhibit the proliferation of vascular smooth muscle. The latter finding suggests that decreased NO release from injured endothelium causes disinhibition of the proliferation of vascular smooth muscle.

L-arginine inhibits balloon catheter-induced intimal hyperplasia.\textsuperscript{63} These observations suggest a preventive or ameliorative role for NO in atherosclerosis. However, LDL is modified by NO and superoxide anion,\textsuperscript{64} and becomes a ligand for the scavenger receptor of macrophages, suggesting that coexistence of NO and superoxide radicals may exacerbate the disease. Again, this issue is relevant to the redox state of NO, as discussed in the section on neuronal death.

Scavenging of NO by advanced glycosylation end products and oxidized lipoproteins may contribute to defective vasoregulation in atherosclerosis.\textsuperscript{65}
Cerebral vasospasm

Vasospasm following subarachnoid hemorrhage may involve NO. Oxyhemoglobin, an inactivator of NO, is potentially one of the major factors responsible for cerebral vasospasm following subarachnoid hemorrhage. Thus, intracisternal injections of hemoglobin in the pig induce concentration-dependent vasoconstriction of intrathecal arteries. Since NO is a potent inhibitor of platelet aggregation, in the absence of NO platelets would be expected to aggregate and release vasoconstrictors such as serotonin which would in turn contribute to the cerebral vasospasm.

Dilated cardiomyopathy

In patients with dilated cardiomyopathy, iNOS was markedly induced. The excessive formation of NO may contribute to the dilation of cardiac muscles, since NO plays a role in inhibiting cardiac muscle contractility.

Peripheral and Central Nervous Systems

NO satisfies several criteria for a neurotransmitter, including occurrence of the synthetic enzyme in the relevant neurons, mimicking by NO of the effects of physiologic nerve stimulation, and blockade of the effects of nerve stimulation after inhibition of NO synthesis. However, NO is an atypical neurotransmitter candidate. Most neurotransmitters are stored in synaptic vesicles, released by fusion with the synaptic membranes. In contrast, electron microscopic immunohistochemistry shows that in myenteric plexus nerve terminals NO does not occur in synaptic vesicles or in association with the plasma membrane, but rather appears to be largely cytoplasmic. NO is synthesized on demand and diffuses freely out of neurons. In postsynaptic cells its only known receptor or acceptor is the iron in guanylate cyclase. Recent evidence suggests that in addition to iron, a sulfhydryl moiety may be an acceptor of nitrogen monoxide in the nitrosonium cation form. Like other neurotransmitters, NO release depends on Ca2+ influx into nerve terminals. The calcium/calmodulin-dependent enzyme, NOS, is activated by a increase in cytosolic Ca2+. In the gastrointestinal tract, NOS is colocalized with vasoactive intestinal polypeptide. No likely functions as the major inhibitory neurotransmitter of the gut.

Cerebral artery

Smooth muscles of cerebral arteries are innervated by nerves whose electrical stimulation produces relaxation not mediated by adrenergic or cholinergic transmitters. Many of these nonadrenergic noncholinergic (NANC) nerves generate and release NO. Immunohistochemical evidence has demonstrated the existence of eNOS in NANC nerves. Blood vessels are also innervated by NANC nerves and many of these NANC neurons also release NO as a neurotransmitter.

NOS immunoreactivity occurs in adventitial nerve fibers of cerebral blood vessels. NOS inhibitors block neurally induced vasodilation in cerebral arteries. NO appears to be a major regulator of local blood flow. In cerebral arteries these neurons are parasympathetic, their cell bodies being located in the sphenopalatine ganglia.

Analgesia

Peripheral as well as central effects of morphine are inhibited by an NOS inhibitor. L-arginine administration causes analgesia and this L-arginine effect may be mediated through NO.

Corpus cavernosum

Nitric oxide and cyclic GMP formation in response to electrical field stimulation cause relaxation of corpus cavernosum smooth muscle, suggesting that penile erection may be mediated by NO generated in response to NANC neurotransmission. In intact rats, physiologic stimulation of the pelvic nerves leads to penile erection, which is abolished by small doses of NOS inhibitors. NOS is localized to penile neurons innervating the corpora cavernosa and to neuronal plexuses in the adventitial layer of penile arteries. It is likely that in humans, NO is essential for penile erection.

The nitric oxide donor linsidomine chloride hydrate (SIN-1) was administered to patients with erectile dysfunction. Out of 63 patients, 29 had a full, 21 an almost full and 13 a moderate reaction to 1 mg SIN-1.

Central nervous system

Possible functions for NO as a neurotransmitter in the central nervous system are suggested by the localization of NOS in the brain. NOS occurs in discrete neuronal populations including basket and granule cells of the cerebellum, the cerebral cortex, hippocampus and corpus striatum. NO containing neurons comprise 1 to 2% of the neuronal population. This situation is comparable to that of catecholamine containing neurons which comprise only 1%, but whose processes ramify sufficiently in the cerebral cortex to contact most cells.

Retinal gap junction

L-arginine inhibited retinal gap junctions and this effect was reversed by an NOS inhibitor, L-NMMA.
suggesting a role for NO in the gap junction. It has been demonstrated that NOS is present in the retina.

**Blood pressure regulation**

NO decreases central sympathetic outflow and mediates L-glutamate-elicited decreases in blood pressure and heart rate through baroreceptor-like reflexes in the nucleus tractus solitarius.

**Long term depression and potentiation**

NO may be a mediator of long-term synaptic depression (LTD) in the cerebellum and long-term potentiation of synaptic transmission (LTP) in the hippocampus. Both LTD and LTP are considered to function in the mechanism of experience-driven synaptic remodelling that may underlie learning and memory.

Inhibiting synthesis of the putative messenger, nitric oxide, results in amnesia in a passive avoidance task in the chick. On the other hand, activation of N-methyl-D-aspartic acid (NMDA) receptors before tetanic stimulation blocks LTP in CA1 of the hippocampus. This NMDA-mediated inhibition of LTP can be reversed by an NOS inhibitor and mimicked by sodium nitroprusside. These results indicate that the timing of NO release relative to high-frequency activation may be an important determinant of LTP generation. Therefore, NO may play a positive or a negative modulatory role in LTP.

NMDA receptor stimulation activates nitric oxide synthesis from L-arginine in rat brain slices. Purification and cloning of cerebellar NOS, followed by the use of an antibody to identify cNOS-related antigens in the cerebellum strengthened the view that NO may mediate neuronal responses to excitatory amino acids. A current view is that glutamate released from certain presynaptic neurons elicits a calcium transient in the postsynaptic neuron, activating its NOS. Formed NO in turn diffuses from the postsynaptic neuron in a retrograde manner to reach the presynaptic neuron.

There is no convincing evidence, however, for the existence of NOS in postsynaptic neurons. Instead, it has been shown that astrocytes have cNOS-like activity and iNOS induced by immunological stimuli. The demonstration of cNOS activity in astrocytes is not convincing, because potential LPS contamination was not controlled such that the apparent cNOS activity may in fact have been iNOS activity. It is known that contamination with undefined concentrations of LPS with experimental systems may induce NO.

In LTD, stimulation of climbing fibers and parallel fibers results in diminished synaptic efficacy of parallel fiber-Purkinje cell synapses. Climbing fiber stimulation releases NO, and NOS inhibitors block LTD. Methylene blue, a guanylate cyclase inhibitor, blocks of LTD in the cerebellum, suggesting involvement of NO-cyclic GMP system in LTD.

**Arousal mechanisms**

Pretreatment with L-NAME, given systemically or intracerebroventriculally, significantly reduced the sound-evoked arousal response. Therefore, NO may be one of substances necessary for maintaining the aroused state.

**Feeding behavior**

Acute or repeated administration of an NOS inhibitor, N^G^-nitro-L-arginine, reduced food intake and body weight in both genetically obese and lean rats. Similarly, an inhibitor of NOS, L-N^G^-nitro-arginine methyl ester, produces an L-arginine reversible decrease in food intake in mice. Therefore, NO may play a stimulating role in food intake in animals.

**Neuronal death**

Excessive glutamate release from resistant neurons has been implicated in the death of surrounding cells in ischemic brain injury. Glutamate stimulates NO through NMDA type receptors. NMDA receptor activation triggers the influx of Ca^{2+} into neurons. NO is activated by Ca^{2+} binding to the calmodulin associated with the enzyme. Formed NO diffuses into adjacent cells, producing injury. The surviving neurons are the same subpopulation of NADPH-positive cells. Since cNOS is an NADPH diaphorase, it is possible that cNOS-containing neurons kill cNOS-negative cells. This contention is supported by the ability of an NOS inhibitor to block the neurotoxic actions of glutamate. However, despite the strong evidence for NO-mediated neurotoxicity, in some studies it also seems to be neuroprotective. The neuroprotective action may be explained by observations that NO can nitrosylate the NMDA receptor, blocking glutamate neurotransmission.

The paradox of NO being both neuroprotective and neurodestructive was resolved by Lipton et al (1993). It has become clear that the congener of nitrogen monoxide, NO, should be taken into account. NO-mediated neurotoxicity is generated, in part, by reaction with superoxide anion (O_2^-), apparently leading to formation of peroxynitrite (ONO'O^-), and not by NO alone. In contrast, the neuroprotective effects of NO result from down regulation of NMDA-receptor activity by reaction with thiol group(s) of the receptor's redox modulatory site. This reaction is not mediated by NO itself, but by NO^+ causing S-nitrosylation of NMDA receptor thiol. These results are at least partially...
consistent with the observation that NOS neurons, which are resistant to glutamate toxicity, are rich in manganese superoxide dismutase \(^{109}\) which could protect NO\(^+\) from superoxide anion.

**Ischemic injury**

In the heart, as well as the brain, the roles of NO in ischemia-associated injury are controversial. It has been reported that addition of NO or L-arginine diminishes infarct size after reperfusion. \(^{110}\) An NOS inhibitor, NO-nitro-L-arginine methyl ester (L-NAME), exacerbated the reperfusion injury. \(^{111}\) Since NO production is increased during reperfusion, as shown by the same author as well as others, \(^{112}\) this suggests that NO plays a role in ameliorating reperfusion injury. Another report, however, describes the ameliorative effect of an NOS inhibitor, L-NAME, on reperfusion injury. \(^{113}\)

**Allergic encephalitis**

Both infection with one of several viruses known to cause neural pathology and induction of experimental allergic encephalitis can cause the appearance of pathological change and also the induction of NOS. \(^{114}\) Identified sources of iNOS in the brain include astrocytes and microglia. \(^{97,115}\)

NO may be responsible, in part, for encephalitis in patients with acquired immunodeficiency syndrome (AIDS). The neural toxicity caused by the coat protein to human immunodeficiency virus type 1 (HIV-1), the infectious agent in AIDS, may be mediated through NO. \(^{116}\) The coat protein gp120 of HIV-1 exerts an agonistic action on the glutamate receptor, which is well known to generate NO and to cause neuronal death. Small heat-stable toxins secreted by HIV infected mononuclear phagocytes also appear to act via NMDA receptors. \(^{117}\)

**Convulsion**

Cyclic GMP has long been suggested to play a role in seizures and the cyclic GMP levels increased in several brain regions before the onset of seizures. \(^{118}\) Excitatory amino acid-mediated convulsant activity may occur through the subsequent activation of NOS. Thus, intracerebroventricular injections of exogenous L-arginine induced behavioral and electroencephalographic activation. \(^{119}\) L-Arginine also produces proconvulsant effects in rats treated with subconvulsive doses of NMDA, and these effects are prevented by an NOS inhibitor. Harmaline-induced tremor may involve NO, since harmaline administration to rats activates climbing fibers within the cerebellum, releasing glutamate with the subsequent formation of NO. \(^{120,121}\)

**Pancreatic Islet Function**

Antibody to constitutive NOS stains islet B cells, \(^{122}\) and the substrate of NOS, L-arginine, stimulates insulin release from the islets. \(^{123}\) This raises the possibility that, in diabetes mellitus, the L-arginine-NO pathway is impaired. However, the issue is not straightforward, since at a lower concentration NO plays a regulatory role and excessive NO apparently impairs islet functions.

Islet cell death during the development of Type 1 (insulin-dependent) diabetes mellitus has been thought to be mediated in part by oxygen radicals and interleukin 1. Cytokines induce iNOS in islets and inhibit insulin release \textit{in vitro}. \(^{124}\) Recent evidence indicates that interleukin-1-induced islet cell lysis is completely abolished when NO synthesis has been prevented. \(^{125}\) The induction of diabetes mellitus by administration of streptozotocin to animals seems to be associated with production of islet iNOS. The administration of an NOS inhibitor diminished both insulinitis and hyperglycemia. \(^{126}\) An NO donor, nitroprusside, was shown to be cytotoxic against a murine beta-cell line. \(^{127}\) The sources of iNOS in islets include macrophages and endothelium. We can speculate as to the mechanism of NO cytotoxicity in islets: nitric oxide, which is induced by interleukin 1 and other stimuli, reacts with oxygen radicals, resulting in the formation of a highly toxic compound, peroxynitrite.

Impaired endothelium-dependent relaxation has been observed in blood vessels of genetically diabetic rats. \(^{128}\) In drug-induced diabetic rats, results are controversial. \(^{129,130}\) In alloxan-induced diabetes, neurogenic and endothelium-mediated relaxation of rabbit corpus cavernosum smooth muscle are both impaired. \(^{131}\) Since NO is an essential molecule for neurogenic penile erection, as mentioned earlier, this may one mechanism underlying the impotence associated with diabetes mellitus.

Scavenging of NO by advanced glycosylation end products may also contribute to defective vasoregulation in diabetes. \(^{132}\)

**Immune Systems**

NO mediates cell death in cytostasis of bacteria and other microorganisms in macrophages-mediated cytotoxicity. \(^{133,134}\) NO inhibits several enzyme activities in mitochondrial electron transport and the rate-limiting enzyme in DNA replication, ribonucleotide reductase. \(^{135}\) All of these enzymes have a catalytically active nonheme iron-sulfur complex that is an NO target.

The suppressive effect of activated macrophages on the proliferative responses of lymphocytes to mitogens or antigens can be attributed in part to NO. \(^{136-138}\) It has been demonstrated that L-arginine administration to humans stimulates the activities of natural killer cells. \(^{139}\)
L-arginine stimulates wound healing and immune function in elderly human subjects.\textsuperscript{140} Kupfer cells, activated by close contact with tumor cells, release NO which in turn damages the tumor cells by inhibiting mitochondrial energization.\textsuperscript{141}

A significant rise in serum NO\textsubscript{2}/NO\textsubscript{3} levels in allograft animals preceded the onset of clinical signs of rejection or graft-versus-host disease.\textsuperscript{142} Treatment of allograft recipients with immunosuppressive agents inhibited NO production. These findings suggest that serum NO\textsubscript{2}/NO\textsubscript{3} might be a useful early serum marker of the initiation of a rejection reaction or graft-versus-host disease even in the absence of other functional markers.

**Digestive Tract**

The apparent involvement of NO in NANC neurotransmission in the many regions of the digestive tract in animals\textsuperscript{143-145} and humans\textsuperscript{146} has been shown.

Nitric oxide is involved in the reflex relaxation of the stomach to accommodate food or fluid.\textsuperscript{147} NOS is known to be present in nerves innervating the rat stomach.\textsuperscript{148}

**Hypertrophic pyloric stenosis**

In patients with hypertrophic pyloric stenosis, a lack of nitric oxide synthase in pyloric tissue is responsible for the pylorospasm characteristic of this disease.\textsuperscript{149}

**Conclusion**

Nitroglycerin, an NO-producing drug, was used for the treatment of angina pectoris long before the discovery of endogenous nitric oxide. There are a number of such instances, in which a compound was in clinical use long before its corresponding endogenous substance was identified. Morphine, and atropine, for example, were in widespread use before their respective endogenous substance, opioid peptides and acetylcholine, were known. In contrast to these substances, however, the discovery of nitric oxide prompted several clinical trials with nitric oxide-related compounds in human subjects. In light of the numerous physiological functions of nitric oxide, it is clear that not only nitric oxide generators, but also inhibitors of NO synthase and modifiers of redox state of nitrogen monoxide, potentially have broad clinical applications. Further understanding of the physiology and pathophysiology of nitric oxide will undoubtedly open new therapeutic horizons.

**References**


22. Radomski NW, Palmer RMJ, Moncada S: An L-arginine/nitric


