NITRIC OXIDE

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Not so long ago the role of the vascular endothelium was unknown.It was considered as a monolayer barrier between the blood and the vessel wall. The endothelium is the largest organ in the body and in the last 20 years its active metabolism has been recognized.

In humans, the endothelium has a surface area similar to that of six tennis courts and weights 1 to 1.5kg. Its function is not only the regulation of vasomotor tone but also in modulating the inflammatory response, haemostasis, vascular cell growth and transcellular metabolism (Table 1). Certain pathophysiological conditions such as atheroma, diabetes, hypertension, immunological disease are associated with impaired endothelial function (1).

Antithrombotic substance
Alpha 2-macroglobulin
Antithrombin III
Heparin sulphate
Plasminogen activator
Prostacyclin
Protein C
Metabolized substances
Adenine nucleotides
Adenosine
Angiotensin I and II
Bradykinins
Leukotrienes
Norepinephrine
Prostaglandins
Serotonin
Substance P
Substances promoting thrombosis or activating the coagulation system
Fibronectin
Platelet aggregating factor
Thromboplastin
Thromboxane A2
Von Willebrand factor
Secreted substances
Endothelial-derived constricting factors
Endothelial-derived hyperpolarizing factors
Endothelial-derived relaxing factors
Prostacyclin

Table1: Biologically active substances produced or metabolized by the vascular endothelium

The story about nitric oxide (NO) began in 1914 when Sir Henry Dale first observed that intravenous injection of Ach increased blood flow in the arteries of rabbit ear and dilated vessels in vivo. Investigators attempted to confirm Ach's action invitro ,but without success. The mystery for researchers was that the isolated rabbit artery contracted, but didn't relax in response to Ach in vitro.In 1962 a paper appeared by Jelliffe which described blood vessel relaxation with Ach. He used a ring preparation of rabbit aorta in place of helical strip. Furchott repeated these experiments and confirmed that Ach relaxes the ring preparation but not the helical strip preparation. Furchott identified that endothelium had been injured during preparation of the helical strip. He decided that Ach-induced relaxation is endothelium dependent. So he named this substance Endothelial Derived Relaxing Factor. For several years attemps to characterize EDRF were unsuccessful. Pharmacological similarities between EDRF and nitrodilator drugs, Moncada in 1987 presented evidence that EDRF would be identical to NO. Now EDRF has been identified definitively as either NO or close derivatives that releases NO. The exponential and cumulative knowledge of NO and its implications for physiology and pathology led to the naming of NO as "Molecule of the year for 1992" (Fig. 1) (2, 3).

In the ambient air NO concentration changes according to the medium. Heavy traffic increases its basal level. NO is exhausted by the supersonic aircrafts and motorcars. Its recycling is shown in Figure 2.

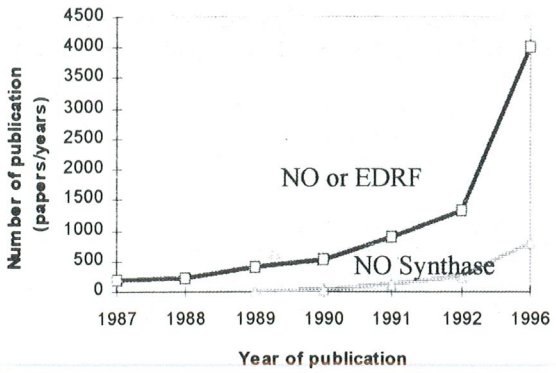


Fig. 1: The annual rate of publications on nitric oxide or EDRF and on NO synthases.

$\cdot NO + O_3 \rightarrow O_2 + NO_2$

$NO_2 + O \rightarrow O_2 + \cdot NO$ (This effect is dangerous for ozone layer (4)).

Fig. 2 : NO recycling.

NO may be the product of the following reaction in the ambient air:

$N_2 + O_2 \rightarrow \rightarrow$ (1000C°) NO

Properties of nitric oxide depend on its interrelated redox forms:

Neutral nitric oxide --> (NO•)

Nitrosonium cation --> (NO+)

with the removal of an electron from its outer orbit

Nitrosyl anion --> (NO-)

with the addition of an electron to its outer orbit

Of course neutral nitric oxide(NO°) is a free radical and different from the anaesthetic gas nitrous oxide (N_2O) and may react with other free radicals. It reacts with 0_2. to form peroxynitrite (00N0-) a powerful oxidant which can cause tissue damage after its protonization and represents the ugly side of NO. Generally 1-5 % of oxygen consumption is estimated to be reduced to superoxide and high NO levels competes with SOD to react with O2. Peroxynitrite is protonated to form strong oxidant peroxynitrous acid and it resembles OH in its reactions (5, 6).

NO is synthesized from L-arginine by NO synthase(NOS) which exists in several cell specific isoforms (Fig. 3 and Table 2).

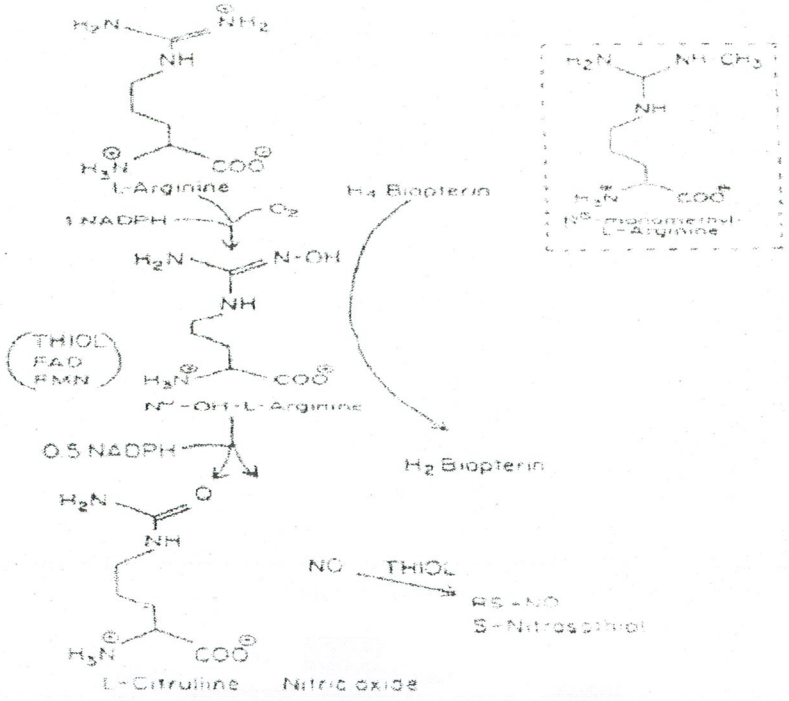


Fig - 3 : Procedure of NO synthesis.

Type	*Cosubstrates.*	*Regulated by*	*M of Denatured Protein (SDS-PAGE)*	*Present*
Ia	NADPH,BH4, FAD/FMN	Ca^{2+}/calmodulin	155 kDa	Brain, cerebell., NIE-115 neuroblast. Cells
Ib	NADPH	Ca^{2+}/calmodulin	Unknown	Endothelial cells
Ic	NADPH,BH4,FAD	Ca^{2+} (not calmodulin)	150 kDa	Neutrophils
II	NADPH,BH4,FAD/FMN, GSH (thiols)	Unknown	125 to 135 kDa 150 kDa	Macrophages
III	NADPH,BH4	Ca^{2+}/calmodulin	135 kDa	Endothelial cells
IV	NADPH	Unknown	Unknown	Macrophages

ec NOS III, nc NOS I, i NOS II

Table 2 : Isoforms of NOS.

NOSs fall in to two distinct categories: constitutive(cNOS: ecNOS and ncNOS) and inducible(iNOS) enzymes each having FAD, FMN, THB, haem prosthetic groups. NOS II but not NOS I and NOS III activity is largely (human) or completely (mouse and rat) Ca(2+)-independent. Expression is enhanced by e.g. estrogens (for NOS I and III), shear stress, TGF-beta 1, and (in certain endothelial cells) high glucose (for NOS III) (7).

Many agonists cause the release of NO (Table 3 and Fig 4).

Physiological	
Change in flow or shear stress	
Change in oxygen tension	
Vasoactive substances	
Acetylcholine	Calcium ionophore (A23187)
Adenosine diphosphate	Cholecystokinin
Adenosine triphosphate	Vasoactive intestinal peptide
Bradykinin	Trombin
Calcitonin gene-related peptide	Substance P
Histamine	Serotonin
Noradrenaline	

Table 3 : Stimulants of EDRF release.

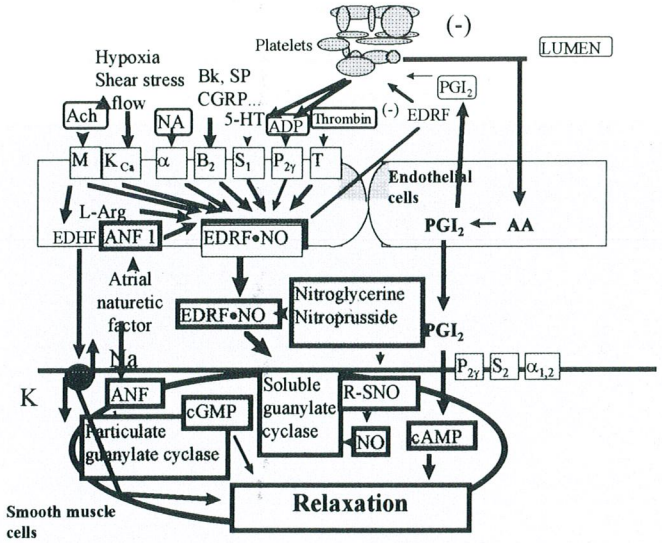


Fig. 4 : Stimulants and receptors for EDRF/NO release.

NO synthesis is inhibited by the competitive inhibitors. These are: Monomethyl-L-arginine (L-NMMA), asymmetric dimethyl-L-arginine (L-ADMA), nitro-L-arginine(L-NNA), nitro-L-arginine methyl ester(L-NAME)

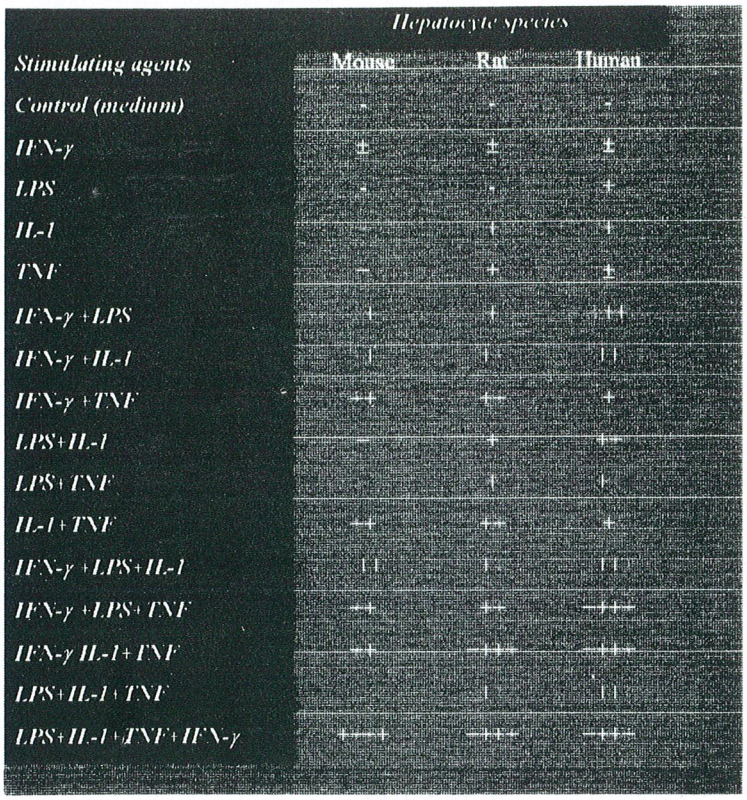
cNOS needs the Ca-CaM for its activation but iNOS is Ca independent.

iNOS stimulators: LPS, TNF-α, IFN-γ,IL-1, IL-2 (Table 4).

iNOS is inhibited by glucocorticoids, dexamethasone, progesterone, TGF-α, IL-4, EGF, PDGF, IL-8, cycloheximide, actinomycine D (8).

NO can exert feedback inhibition on cNOS probably interacting with NOS hem. NO or its stable derivatives stimulate guanylate cyclase by binding of its hem group in smooth muscle cells and increase the cGMP levels. cGMP reduces the intracellular Ca and opens Ca++ activated K+ channels. K efflux leads to hyperpolarization and causes relaxation (9).

The molecular targets of NO are hem proteins, Fe-S proteins such as aconitase and thiol groups such as gliseraldehyde-3-phosphate



Stimulating agents	Hepatocyte species: Mouse	Rat	Human
Control (medium)	-	-	-
IFN-γ	±	±	±
LPS	-	-	+
IL-1	-	+	+
TNF	-	+	±
IFN-γ +LPS	+	+	+++
IFN-γ +IL-1	+	++	++
IFN-γ +TNF	++	++	+
LPS+IL-1	-	+	++
LPS+TNF	-	+	+
IL-1+TNF	++	++	+
IFN-γ +LPS+IL-1	++	++	+++
IFN-γ +LPS+TNF	++	++	+++
IFN-γ IL-1+TNF	++	+++	+++
LPS+IL-1+TNF	+	+	++
LPS+IL-1+TNF+IFN-γ	+++	+++	+++

Table 4 : iNOS stimulators.

dehydrogenase and the superoxide radicals.

Th1 cells produce NO and this secreted NO exerts negative feedback effect on the same cell to inhibit the production of IL-2 and IFN-Ɣ.

Physiologically, the formation of NO in the endothelial cells may be elevated by an increased blood flow. The increased shear (viscous drag) and pulse pressure activate mechanoreceptors on the surface of endothelial cells and promote the activity of NOS and consequantli yields more NO (10).

The reaction of organic peroxyl radicals with NO has been described:

ROO· + ·NO --> -->ROONO. This organic peroxynitrite(ROONO) is not decomposed to form free radicals. NO also inhibits LDL peroxidation.

In intact cells cyclo-oxygenase activity is enhanced by NO (11).

S-nitrosothiols (e.g. glutathione S-NO, cysteine S-NO or serum albumine S-NO) are able to inhibit platelet aggregation and adhesion to endothelial cells by elevating cGMP (11).

Endothelin-1 acts on specific receptors on the endothelium to increase the release of nitric oxide and prostacyclin, while nitric oxide depresses the production and/or release of endothelin-1 from endothelial cells. Endothelial cells express ET(B) receptors linked to the formation of nitric oxide and/or prostacyclin formation (12,13,14).

NO diffusing luminally from the vascular wall is rapidly bound to the oxygenated or non-oxygenated hemoglobin. NO met by HbO2 is

rapidly converted to nitrate ion, leaving methemoglobin whilst NO unifying with non-oxygenated Hb forms the rather stable nitrosohemoglobin molecule(HbNO). HbNO may be converted to nitrate and methemoglobin when oxygen tension increases (15,16).

Fish oil, vitamin E and SOD have been shown to promote endothelium -dependent vasodilatation, probably by prolonging NO survival in the vessel wall.In general arteries and arterioles produce more NO than venes and venules. In addition, NO plays a role in the development of reactive hyperemia (15).

Adhesion molecules play a very important role in reperfusion injury. There are three families of cell adhesive molecules (Table 5).

Inhibitors of NOS lead to increased expression of CD11a,b,c /CD18 on neutrophils and cause attachment of the cells to the vessel wall. NO

Selectins:

P Selectins L Selectins E Selectins

β_2 integrins:

CD 11 $_{a,b,c}$ CD18

Immunglobuline superfamily:

ICAM-1 VCAM-1 PECAM-1

Table 5 : Adhesion Molecules.

inhibits the up-regulation of P-selectin and decreases the surface expression of ICAM-1.Thus NO inhibits the reperfusion injury (17).

GP IIb IIIa (fibrinogen receptors up-regulated by activated platelets) are sensitive to NO. NO decreases the P-selectin expression and GP IIb IIIa upregulation on platelets (18).

Nitroglycerin, isosorbide dinitrate, sodium nitroprusside and other nitrate vasodilators are ultimately biotransformed to NO.

Immunocytochemical and histochemical studies of cat and rat carotid bodies have revealed a plexus of nitric oxide synthase (NOS)-positive nerve fibers associated with lobules of chemosensory type I cells as well as with the carotid body vasculature.

NO is vital in the developing kidney to maintain normal physiological function. nitric oxide is produced in renal arteries, macula densa, glomeruli, and tubules by different NO-synthases. Nitric oxide contributes to physiological regulation of renal blood flow, renal autoregulation, tubuloglomerular feedback, renin release, pressure natriuresis, and tubular function. Increases in arterial pressure increase endothelial nitric oxide formation which inhibits sodium reabsorption via direct effects on the tubules (19, 20).

NO produces a time dependent change in renin secretion with an initial inhibition of renin release. After longer exposure to NO, stimulation of renin secretion occurs.

Nitric oxide synthase (NOS), has been shown to be localized in nerve fibers of the detrusor, trigone, and urethra (21). NO also inhibits smooth muscle migration and proliferation

NGF induces different forms of NOS in neuronal cells.

Pretreatment with L-NAME, given systematically or intraventricularly significantly reduced the sound-evoked arousal response, suggesting a role for NO.

Neuronal NOS have been detected in the retina and choroid with the highest concentration in fovea. Neuronal NOS may be responsible for producing nitric oxide in photoreceptors and bipolar cells. Nitric oxide stimulates guanylate cyclase of photoreceptor rod cells and increases calcium channel currents. In the retina of cats, NOS inhibition impairs phototransduction as assessed by the electroretinogram. Inducible nitric oxide synthase, found in Muller cells and in retinal pigment epithelium, may be involved in normal phagocytosis of the retinal outer segment, in infectious and ischemic processes, and in the pathogenesis of diabetic retinopathy. Nitric oxide contributes to basal tone in the retinal circulation. To date, findings are conflicting with respect to its role in retinal autoregulation. During glucose and oxygen deprivation, nitric oxide may increase blood flow and prevent platelet aggregation, but it may also mediate the toxic effects of excitatory amino acid release. In beagles NO production by systemic administration of an NOS inhibitor led to a decrease in blood flow to the choroid, ciliary body and iris (22, 23).

In the stomach Calcitonin gene-related peptide(CGRP) stimulates the production of NO which relaxes the adjacent vascular smooth muscle cells. The resulting vasodilation increases the delivery of bicarbonate to the epithelium where it can neutralize acid and also NO inhibits the acid secretion through suppression of histamine release from mast cells. In addition to eNOS, iNOS has been found in enteric neurons and in contrast to the predominant suggestion it can exert cytoprotective effects. NO also increases the cyclooxygenase activity (24).

Chewing, saliva, and gastric acidity support gastric NO release, important for mucosal blood flow, gastrointestinal (GI) motility, mucus formation, and bacteriostasis. An oral supply of NO-donating substances and chewing of nitrate-rich food, such as lettuce or spinach, can be useful (25).

NO is the main mediator of penile erection in men and particularly in rats in the nerve terminals. Penile NOS activity is decreased in certain conditions such as decreased serum testosterone levels, extreme senescence, castration, diabetes types I and II, adrenalectomy, hypophysectomy. Only nNOS type has been demonstrated in human penis. Chronically exposed to cigarette smoking, there is a pronounced loss of penile NOS activity. NOS activity and eNOS has been found in Sertoli, Leydig cells and it affects the androgen production. In addition It is found in prostate and seminal vesicles suggesting possible role in sperm maturation (26).

Septic shock is associated with bacterial LPS and release of numerous cytokines. The NOS inhibitor, L-NMMA reversed the hypotension caused by bacterial LPS and tumor necrosis factor.

Peripheral as well as central effects of morphine are inhibited by an NOS inhibitor , L-arginine administration causes analgesia.

NOS has been found in discrete neuronal populations including basket and granule cells of the cerebellum, the cerebral cortex, hippocampus and corpus striatum.

In the ovary, gonadotropins, estrogens, growth hormone, growth factors (IGF I, EGF/TGF-α, basic FGF), cytokine (interleukin-1ß) and nitric oxide act in concert to ensure the survival of preovulatory follicles (27).

Nitric oxide (NO) is a potent smooth muscle relaxant in blood vessels, the gastrointestinal tract and the respiratory system. Recent evidence has shown that NO has a relaxant (tocolytic) effect on myometrium. NO is produced within the female genital tract during pregnancy, and a reduction in NO synthesis may be involved in the initiation of parturition. Higher NOS activities were seen in first trimester placental villi than at term. An impairment of NO metabolism occurred in placental villi from pre-eclamptic and growth-retarded pregnancies. Smoking also results in decreased NOS activities in the placental villi, suggesting that problems attributed to smoking during pregnancy could be linked to NO metabolism Furthermore, the administration of NO donors may be useful in inhibiting uterine contractions in situations where such activity is unwanted, e.g., in preterm labor. NO is also produced in the myometrium in the nonpregnant state, and has potential roles in the facilitation of

implantation and the prevention of dysmenorrhoea (28).

Estrogens can modulate vascular function by increasing nitric oxide production via stimulation of endothelial nitric oxide synthase (eNOS) and decreasing endothelin-1 levels in vivo (29).

Topical NO donors have crucial role in the treatment of fungal and other infectious diseases. L-arginine stimulates wound healing, and NK cell activity

Interactions between metastasizing tumor cells and host cells in target organs determine the outcome of metastasis. Endothelial cells can contribute to host anti-metastatic responses, e.g. by production of the cytotoxic molecule nitric oxide (NO) from arginine with the help of the inducible nitric oxide synthase (iNOS)(30).

Involvement of nitric oxide (NO) in the regulation of mineralised tissue function, and the potential role of NO in maintenance of the ligament space is considered(31).

In the outer hair cells NOS I and III reactivity and in the inner hair cells NOS III reactivity were observed. It has been demonstrated that the high concentrations of NO elevates hearing treshold in the cochlea (32).

The urinary nitrite/nitrate(stable metabolites of NO) excretion is highest in the young children and decrease in an age-dependent manner to reach constant levels at about 12 years of age in both sexes (33).

NO inhibits MHC class II but not MHC class I expression (34).

Both constitutive and inducible forms of NO synthase are expressed by bone-derived cells, and cytokines such as interleukin-1 (IL-1), tumor necrosis factor (TNF), and interferon gamma (IFN-gamma), are potent stimulators of NO production. When combined with other cytokines, IFN-gamma markedly induces NO production, which suppresses osteoclast formation and activity of mature osteoclasts. This "superinduction" of NO is largely responsible for the selective inhibitory effect of IFN-gamma on cytokine-induced bone resorption. High concentrations of NO are also inhibitory for cells of the osteoblast lineage, and NO production appears to be partly responsible for the inhibitory effects of cytokines on osteoblast proliferation. At lower concentrations, however, NO has different effects. Moderate induction of NO potentiates bone resorption, and the constitutive production of NO at low concentrations promotes the proliferation of osteoblast-like cells and modulates osteoblast function. NO therefore appears to be an important regulatory molecule in bone with effects on cells of the osteoblast and osteoclast lineage and represents one of the molecules produced by osteoblasts which directly regulate osteoclastic activity. Stimulation of NO production in bone by proinflammatory cytokines raises the possibility that NO may be involved as a mediator of bone disease in conditions associated with cytokine activation, such as rheumatoid arthritis, tumor associated osteolysis, and postmenopausal osteoporosis (35).

Potassium currents through calcium-activated channels in vascular smooth muscle cells are increased in response to NO or upon exposure to cGMP-dependent protein kinase (36).

The microcirculation undergoes a profound degree of endothelial dysfunction within minutes (i.e., 2.5 to 5 min) following reperfussion of ischaemic vasculature. This has been documented in the coronary and mesenteric microcirculation. The endothelial dysfunction is characterized by a loss in basal and agonist-mediated nitric oxide (NO) produced by the vascular endothelium. The loss of NO results in upregulation of cell adhesion molecules (CAMs) particularly P-selectin 10-20 min following reperfusion. Thus, CAM upregulation renders the endothelium sticky, and a marked degree of leukocyte adherence (particularly neutrophils) occurs 20 min following reperfusion. This enhanced involvement of neutrophils leads to neutrophil infiltration into the underlying tissue (e.g., myocardium) within 2-3 h of reperfusion. The infiltration of neutrophils leads to reperfusion injury (i.e., necrosis) which is significant at 3 h but becomes profound at 4.5 h following reperfusion. Cardiac necrosis can be significantly attenuated by treatment with NO, an organic NO donor, L-arginine, or specific blockers of CAMs given just prior to reperfusion (37).

The cytokine-inducible nitric oxide synthase (NOS) seems to mainly be implicated in the cytotoxic activity of almost all the effector cells involved in tumor cell killing. The cytotoxic actions of NO against tumor cells appear to be

related mainly to inhibition of several heme-containing enzymes of the mitochondrial electron transport complex and the citric acid cycle (38)

Nitric oxide has been detected in the exhaled air of several animal species including humans, and increased levels are found in patients with inflammatory airways diseases such as asthma and bronchiectasis. Nitric oxide has a selective suppressive effect on the Th1 subset of helper T cells (39).

Nitric oxide (NO) is a critical mediator of a variety of biological functions. A range of micro-organisms, including viruses, bacteria, protozoa and helminths, is sensitive to NO produced by macrophages activated with gamma-interferon (IFN-gamma) and lipopolysaccharide. In contrast, NO is involved in a number of important immunopathologies, including diabetes, graft-vs-host reaction, rheumatoid arthritis, systemic lupus erythematosus, experimental autoimmune encephalomyelitis and multiple sclerosis. Thus, it is crucial that the synthesis of NO is under tight regulation. This is achieved, in part, through the opposing cytokines produced by T helper 1 (Th1) and Th2 cells. Th1 cells produce IFN-gamma, which is the most powerful inducer of inducible NO synthase (iNOS). In contrast, interleukin 4 is produced by Th2 cells and inhibits the induction of iNOS at the level of transcription. Furthermore, NO is also produced by Th1 cells, whose proliferation can be inhibited by high concentrations of NO. Thus, apart from being a mediator of Th1/Th2 interaction, NO may also be an important self-regulatory molecule that prevents the over-expansion of Th1 cells which are implicated in a range of severe immunopathologies (40).

Expression of iNOS in tumor cells is associated with apoptosis, suppression of tumorigenicity, abrogation of metastasis (41).

Endogenously generated or exogenously applied NO causes DNA cleavage after endonuclease activation. NO-mediated accumulation of the tumor suppressor p53 precedes apoptotic cell death (42).

Oxygen-radicals are required for the synthesis of nitric oxide by NO synthase as demonstrated by inhibition of NO formation by oxygen-radical scavengers (43).

Nitric oxide (NO) as a neurotransmitter acting as a vasodilator agent is reported in canine cerebral arteries (44).

These observations clearly indicate that NO inhibits NOS activity and that nNOS and eNOS are more sensitive than iNOS to the inhibitory action of NO. Not only exogenously added NO but also enzymatically generated NO inhibits the activity of nNOS and eNOS. The mechanism by which NO inhibits NOS appears to involve the heme iron prosthetic group of NOS. Moreover, the oxidation state of the heme iron is critical in determining the magnitude of inhibition of NOS by NO. Conditions that favor the higher oxidation state of FeIII markedly increase the inhibitory action of NO, whereas conditions that favor the lower oxidation state of FeII markedly decrease the inhibitory action of NO. One of the cofactor roles of tetrahydrobiopterin may be to reduce the negative-feedback effect of NO on NOS by favoring the formation of the ferrous heme state in NOS (45).

Endothelium is an insulin target tissue that exhibits an increase in the release of EDNO(endothelium-derived nitric oxide) in response to insulin. We postulate that the insulin-resistant state of obesity is associated with insulin resistance at the level of the endothelium, reduced EDNOrelease, and impaired vasodilation (46).

Simultaneous exposure of carotenoids to NO2 and light significantly reduced formation of nitrosating intermediates and resulted in the release of nitric oxide (NO) into the gas phase (47).

Nitric oxide signaling is achieved through both cGMP-dependent and cGMP-independent mechanisms. The latter are exemplified by mechanisms inducing accumulation of the tumor suppressor gene p53 and causing apoptotic cell death (48).

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