Janaki Medical College Journal of Medical Sciences (2022); Vol.10 (1):61-67

JMCJMS

Review Article

The Magical Wonders of Nitric Oxide: The Molecule of the Millennium

Varun Malhotra[1], Sunil Chauhan[1], Santosh Wakode[2], Kshitiz Upadhyay-Dhungel[3]

Department of Physiology
AIIMS, Bhopal

Author's Affiliations
[1]Associate Professor, Department of Physiology, AIIMS, Bhopal
[2]HOD, Department of Physiology, AIIMS, Bhopal
[3]Professor Physiology, Janaki Medical College, Nepal

Correspondence to:
Dr. Varun Malhotra
Associate Professor,
Department of Physiology
AIIMS, Bhopal
varun.physiology@aiimsbhopal.edu.in

ABSTRACT

NO is a gas and has a very short half-life (3-5s), as it is highly reactive. No and its products are inactivated through oxidation into nitrite (NO_2^-) and nitrate (NO_3^-) Which is excreted in the urine. NO is regarded as magical molecule which has profound role in regulation of various function in various organs of human body and in health and disease. Here in this review article authors has discussed about its chemistry and metabolism, its discovery, its basic functions, role in pathophysiology of various diseases and its therapeutic implications.

Key Words: NO, eNOS, EDRF,

INTRODUCTION

Chemistry and Metabolism: NO is a gas and has a very short half-life (3-5s), as it is highly reactive. No and its products are inactivated through oxidation into nitrite (NO_2^-) and nitrate (NO_3^-) Which is excreted in the urine [1]. The Plasma and urine concentrations of NO_3^- and cGMP are useful indicators for turnover of NO i.e. its production and excretion [2].

Discovery: An unexpected and exciting discovery was made that relaxation of isolated preparations of rabbit aorta by acetylcholine was eliminated if the intimal surface of preparations was rubbed prior to testing with acetylcholine (Rubbing damaged the endothelium lining cells). This showed that the relaxation by Acetylcholine was not the result of a direct action on the arterial smooth muscle but rather of an indirect action in which Ach acted on muscarinic receptors of the endothelial cells, stimulating these cells to release a factor that in turn acted on the smooth muscle cells to cause relaxation. The relaxing factor was referred to as Endothelium derive Relaxing Factor (EDRF) [3]. Relaxation of arteries by acetylcholine is endothelium dependent [4]. The discovery of the NO – dependent vasodilator tone indicated the existence of an endogenous vasodilator system, the actions of which are imitated by compounds such as glyceryl tri-nitrate and sodium nitroprusside [5, 6].

Synthesis of NO: NO is synthesized from arginine in a reaction catalyzed by nitric oxide

syntheses (NOS) Figure 1. Three isoforms of NOS have been identified. NOS 1 is found in the nervous system, NOS 2 is found in macrophages and other immune cells. And NOS 3 (e-NOS) is found in endothelial cells. NOS 1 and NOS 3 are activated by agents that increase intracellular Ca++ concentration, including the vasodilators acetylcholine and bradykinin. The NOS in immune cells is not induced by Ca++ but is activated by cytokines. NO is synthesized from arginine in endothelial cells and its action via stimulation of soluble guanyl cyclase and generation of cGMP to produce relaxation in vascular smooth muscle cells. The endothelial form of Nitric oxygen Synthase (NOS) is activated by increased intracellular CA++ Concentration, and an increase is produced by acetylcholine (Ach), Bradykinin or shear stress acting on the cell membrane. Thiol, tetrahydrobiopterin, FAD and FMN are the requisite cofactors [7].

E-NOS (usually referred as eNOS): Originally (e-NOS) characterized in aortic endothelium, now is known to we expressed in cardiac myocytes [8], blood platelets [9], hippocampal neurons [10], pulmonary epithelium [11], renal epithelium [12] and other tissues. In the vascular wall eNOS, plays a key role in vasodilation, and may also regulated vascular smooth muscle cell proliferation, platelet adherence and activation, Leucocyte adherence and chemokine production. In cardiac myocytes, e-NOS play a key role in the modulation of autoimmune control of contractility and heart rate. Endothelial NOS (e-NOS) knock out mice are Hypertensive and lack EDRF activity [13].

Functions of NO

NO in Vascular homeostasis: When flow to a tissue is suddenly increased by arteriolar dilation large arteries to the tissue also dilate. The flow –induced dilation is due to local release of NO is termed as post stenotic vasodilation [14] products of platelet aggregation also cause release of NO, and the resulting vasodilation helps keep blood vessels, within an intact endothelium patent. This is in contrast to injured blood vessels, where the endothelium is damaged at the site of injury and platelets therefore aggregate and produce vasoconstriction. NO relaxes vascular smooth muscle cells. NO interferes with the secretion and action of endothelium, a potent vasoconstrictor. These seems to be a balance between endothelium derived vasoconstrictors and vasodilators for normal orderly for of blood.

NO in penile erection: There is good evidence to suggest that penile erection is produced by release of NO with consequent vasodilation and engorgement of the corpora cavernosa inhibitor of cGMP- specific phosphodiesterase acts by inhibiting the inactivation of NO [15].

NO as a neurotransmitter: NO is produced in the brain and is responsible for long term potentiation- LTE and long term depression [16]. In the cerebellum NO is inhibitory and in the hippocampus NO is stimulatory and plays a role in learning and memory. Simultaneous firing of climbing and parallel fibers in Purkinje fiber.

***Other Function of NO*:** NO is necessary for the cytotoxic activity of macrophages, including their ability to kill cancer cells.

Role of NO in Pathophysiology of Diseases

No in Hypertension: When various derivatives of arginine are administered to experimental animals, there is a prompt rise in blood pressure. This suggests that tonic release of NO is necessary to maintain blood pressure. Impaired production of NO has been implicated in several cardiovascular disorders, including Hypertension, vasospasm and atherosclerosis [17]. Elevated Blood pressure is a common condition that may lead

to well defined complications including stroke, congestive heart failure. In addition, Hypertension is a well-known risk factor for development of atherosclerosis. The vast majority of Hypertensive patients have no apparent cause for their elevated blood pressure called essential hypertension. In humans with essential hypertension there is increasing of NO [18]. Other action of NO that relate to the cardiovascular system include inhibition of white cell activation and inhibition of smooth muscle cell proliferation [17, 19]

NO: The Antiatherogenic molecule: Nitroglycerin & other nitro vasodilators that are of great value in the treatment of angina act by stimulating guanylcyclase in the same manner as NO does. NO decreases LDL oxidation and inhibits superoxide (O^{2-}) production by inhibiting NADP reductase activity. These actions are related to the strong "antiatherosclerotic effets" of NO.

Some of the critical events involved in atherogenesis are monocyte adherence and infiltration, plated adherence and aggregation, and proliferation of vascular smooth muscle cells. EDENO has been shown to inhibit each of these end thus NO is an endogeneous and anti-atherogenic molecule [20-21].

NO and Vascular Inflammation: In addition to its vasodilator actions, NO also contributes to the control of platelet aggregation and the regulation of cardiac contractility. These physiological effects of NO are all mediated by activation of soluble guanylate. NO now is also known to be the mediator released in the peripheral nervous system by a widespread network of nerves, previously recognized as noradrenergic and no cholinergic [22]. Evidence suggests that tonic influence of vasodilator nerves is essential for the maintenance of blood supply to brain regions [23].

NO and Pulmonary Hypertension: More than a century ago [24] it was observed that acute alveolar hypoxia produces pulmonary vasoconstriction. Evidence in favor of a role of NO in limiting hypoxic vasoconstriction has come from experiments in isolated rat lungs in which inhibitors of NO activity or NO synthesis [25]. The pulmonary vasodilator effect of inhaled NO is not accompanied by systemic hypotension, as NO that diffuses into bloodstream is inactivated by binding to hemoglobin [26]. NO appears to be important in the regulation of basal pulmonary vascular tone, in mediating the transition from the fetal to the neonatal circulation, in modulating the pulmonary vasoconstriction associated with acute hypoxia and in limiting the pulmonary vascular remodeling that occurs in chronic hypoxia. Inhaled NO has been shown to decrease pulmonary Hypertension selectively in a variety of clinical settings.

Further advances may include improving pulmonary vascular remodeling in chronic forms of pulmonary hypertension by long-term administration of NO, NO donors, and/or PDE inhibitor.

Therapeutics Implications

Heart Failure: Studies of Arginine in patients with chronic heart failure have shown mixed results. Some studies report improved exercise tolerance. There is evidence from several studies that arginine taken by mouth or by injection improves exercise tolerance and blood flow in arteries of the heart. Benefits have been shown in some patients with coronary artery disease and angina. Further research is needed to establish doses that are safe and effective and to compare Arginine with prescription drugs used for the same purposes. However additional studies are needed.

Peripheral Vascular Disease, Claudication: Intermittent claudication is the

leg pain and fatigue that occur with exercise some people with clots in arteries in their legs. A small number of studies suggest that arginine therapy may improve walking distance; Further research is needed before a strong recommendation can be made [28].

Erectile Dysfunction: With the emerging body of evidence indicating the involvement of the NO pathway in multiple aspect s of the penile erectile response, the use of L-arginine supplementation is a promising therapy in erectile dysfunction. Early studies suggest that men with low nitrate or nitrite levels in their urine may find arginine supplements useful for treating erectile dysfunction in aged[27], smokers [28], diabetes [29], hypertension, atherosclerosis [30] and hypogonadism [31] that are associated with reduced production of EDNO. However, it is not clear what doses may be safe and effective in treating this condition.

Hormone Therapy: In postmenopausal women, estrogen improves coronary and systemic endothelium dependent vasomotor responsiveness; an effect associated with increased NO bioactivity [32].

Future Strategies

There are a number of ways of augmenting NO levels locally including the use of authentic NO either as an inhaled gas or as a dissolved gas in solution, organic NO donors, infusion or diet supplementation with L-arginine or BH^4 or its analogues, NOS gene transfer.

Arginine: NO donor arginine has been suggested as a treatment for many conditions, There is supporting evidence that the use of arginine in treating some heart and vascular conditions, erectile dysfunction and migraine headache pain [33] ,improving recovery after operation. There is not enough evidence for the support of use in medical conditions and research is underway in this direction for future therapeutic implications of NO. Accumulating evidence indicates that supplemental administration of L- arginine is sufficient to restore EDNO production in which EDNO is reduced. Scientists have studied arginine (also known as L-arginine) for the above clinical problems [34].

The development of methods of practical L-arginine delivery in the quantities required will likely accelerate in acceptance in routine clinical practice example in erectile dysfunction, coronary artery disease and interstitial cystitis.

Nitrate*:* Nitroglycerine and organic nitrate continue to play a significant beneficial role in cardiovascular medicine. These agents are useful to all of the ischemic cardiac syndromes, usually as adjunctive therapy. There is no better therapy. For treatment of acute episodes of anginal pain or unstable angina. If the corundum of nitrate tolerance can be resolved' nitrates should enjoy an even larger place as part of our therapeutic Armamentarium [35].

Antioxidants: Endothelium dependent vasodilation is impaired in most if not all the factors associated with atherosclerosis. Antioxidants improve endothelium dependent vasodilation in patients with coronary atherosclerosis. Antioxidant therapies also restore endothelium dependent vasodilation in patients with diabetes, hypercholesterolemia, and hypertension and in smokers. Thus, inactivation of NO by oxygen-derived free radicals may be a theme common to atherosclerosis and its risk factors. Antioxidant therapy by improving the bioavailability of NO [36], may not only improve vasomotor function and subsequently reduced adverse cardiovascular events in patients with atherosclerosis, but also has the potential to retard atherogenesis in patients for atherosclerosis.

NO Donors and their Usefulness: There are a wide variety of nitrogenous compounds that can release NO in solution. These NO generating compounds are collectively known as NO donors. The major classes of organic NO donors are syndnonimines (SIN-1), cysteine-containing NO donors (SPM-5185), the nitrates known as organic nitrates widely used for many years (e.g. Nitroglycerine, sodium nitroprusside) [37].

NO is cytoprotective (suppresses endothelial leucocyte interaction) in reperfusion injury. NO donors have been used to reduce platelet deposition and intimal proliferation following angioplasty. By preventing early platelet attachment and activation to the thrombogenic surface, a S-nitrosated albumin coating may reduce the incidence of acute thrombosis and restenosis following angioplasty [38].

NOS Inhibitors & Gene Therapy: Another approach to study the effects of NO in ischemia reperfusion is to inhibit its synthesis by use of inhibitors of NOS. However, detrimental effects have been reported with use of NOS inhibitors as they also have their own direct affects on the vasculature for example contractile dysfunction after the use of L-NAME. Given to the recent rapid progress in technology development and gene therapy the approach of NO gene therapy in areas of cardiovascular disease e.g. using NOS over expression in atherosclerosis & Prinzmetal angina & inhibition of NOS in septicemia induced vasodilation will almost certainly bring new benefits to treatment of these diseases [39].

REFERENCES

1. Tandon Op. Cardiovascular System Chapter 3 in : Synopsis of Human Physiology: Basic and Applied. Part II. P 54-55.
2. Tandon Op, Sircar SS. Nitric oxide & its role in atherosclerosis In Current Adv Athero Researches Vol II Dwivedi (ED) 67-75,1999
3. Cherry PD, Furchgott RF, Zawadzki JU, Jothianandar The role of endothelial cells in the relaxation of isolated arteries by bradykinin. Proc. Natl. Acad. Sci. USA 1983; 79:2106-2110.
4. Rapport KR, Murad F. Agonist- induced endothelium dependent relaxation in thoracic aorta may be mediated through cGMP. Circ Res 1983; 52:352-357.
5. Moncada S, Palmer RM, Higgs EA The discovery of Nitric oxide as the endogenous nitro vasodilator. Hypertension 1988; 12:365-372.
6. Feelisch M, Stamler J. Donors of nitrogen oxides. In: Feelisch M, Stamler JS eds. Methods in Nitric Oxide Research. New York. John Wiley & Sons 1996:71-115.
7. Ganong William F. Cardiovascular Regulatory Mechanisms Section VI Circulation In Review of Medical Physiology. 20th Edition. p 575-576. Chapter 31.
8. Nitric oxide-dependent parasympathetic signaling is due to activation of constitutive endothelium (Type III) nitric oxide synthase in cardiac myocytes. J Biol Chem 1995; 270: 14582-14586.
9. Sase K, Michel T. Expression ofconstitutive nitric oxide synthase in human blood platelets. Life Sci 1995;57;2049-2055
10. Dinnerman JL, Dawson TM, Schell MJ et al. Endothelial nitric oxide synthase localised to hippocampla pyramidal cells: Implications for synaptic plasticity. Proc. Natl. Acad. Sci. USA 1994; 91:4214-4218.
11. Shaul PW, North AJ, Wu LC et al. Endothelial NOS is expressed in in cultured human bronchiolar epithelium. J Clin Inv 1994; 94:2231-2236.
12. Tracey WR, Pollock JS, Murad F et al Identification of a type ID (endothelial-type) particulate NO synthase in LLC-PKl kidney tubular cells. Am J Physiol 1994 (pt1): 122-28.
13. Huang PL, Huang Z, Mashimo H et al. Hypertension in mice lacking the gene for e-NOS. Nature 1995; 375:408-411.
14. Furchgott RF, Vanhoutte PM. Endothelium derived relaxing and contracting factors. FASEB 1989; 3:2007-2018.

15. Klotz T, Mathus MJ, Braun M et al. Effectiveness of oral L- arginine in first line treatment of erectile dysfunction in a controlled cross over study. Urol Int 1999;(4): 220-223.

16. Bon CL, Garthawaite J. On the role of NO in hippocampal LTP. J.Neuroscience 2003Mar:23(5):1941-8.

17. Garog UC, Hassid A. Nitric oxide-generating vasodilators and 8-bromocyclic guanosine monoposphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells of the genital system. In: Moncada S, Higgs EA eds Nitric oxide from L-Arginine: A Bioregulatory System. Amsterdam; Elsvier Science Publishers Bv, 1990:147-164

18. Calver A, Collier J, Moncada S, Vallance P. Effect of local intra-arterial NG-monomethyl-L-arginine in patients with hypertension: the nitric oxide dilator mechanism appears abnormal. J Hypertens. 1992 Sep;10(9):1025-31. PMID: 1328361.

19. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation. 2004 Jun 15;109(23 Suppl 1):III27-32. doi: 10.1161/01.CIR.0000131515.03336.f8. PMID: 15198963.

20. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1):44-84. doi: 10.1016/j.biocel.2006.07.001. Epub 2006 Aug 4. PMID: 16978905.

21. Sharma JN, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. Inflammopharmacology. 2007 Dec;15(6):252-9. doi: 10.1007/s10787-007-0013-x. PMID: 18236016.

22. Tousoulis D, Kampoli AM, Tentolouris C, Papageorgiou N, Stefanadis C. The role of nitric oxide on endothelial function. Curr Vasc Pharmacol. 2012 Jan;10(1):4-18. doi: 10.2174/157016112798829760. PMID: 22112350.

23. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res. 2000 Nov 10;87(10):840-4. doi: 10.1161/01.res.87.10.840. PMID: 11073878.

24. Melikian N, Seddon MD, Casadei B, Chowienczyk PJ, Shah AM. Neuronal nitric oxide synthase and human vascular regulation. Trends Cardiovasc Med. 2009 Nov;19(8):256-62. doi: 10.1016/j.tcm.2010.02.007. PMID: 20447567; PMCID: PMC2984617.

25. Cannon RO 3rd. Role of nitric oxide in cardiovascular disease: focus on the endothelium. Clin Chem. 1998 Aug;44(8 Pt 2):1809-19. Erratum in: Clin Chem 1998 Sep;44(9):2070. PMID: 9702990.

26. Vaiopoulos AG, Marinou K, Christodoulides C, Koutsilieris M. The role of adiponectin in human vascular physiology. International Journal of Cardiology. 2012.

27. Cartledge J, Minhas S, Eardley I. The role of nitric oxide in penile erection. Expert Opin Pharmacother. 2001 Jan;2(1):95-107. doi: 10.1517/14656566.2.1.95. PMID: 11336572.

28. Bachtiar EW, Putri AC, Bachtiar BM. Salivary nitric oxide, Simplified Oral Hygiene Index, and salivary flow rate in smokers and non-smokers: a cross-sectional study. F1000Res. 2019 Oct 11;8:1744. doi: 10.12688/f1000research.20099.2. PMID: 32269757; PMCID: PMC7111499.

29. Shi Y, Vanhoutte PM. Macro- and microvascular endothelial dysfunction in diabetes. J Diabetes. 2017 May;9(5):434-449. doi: 10.1111/1753-0407.12521. Epub 2017 Mar 1. PMID: 28044409.

30. Pinheiro LC, Tanus-Santos JE, Castro MM. The potential of stimulating nitric oxide formation in the treatment of hypertension. Expert Opin Ther Targets. 2017 May;21(5):543-556. doi: 10.1080/14728222.2017.1310840. Epub 2017 Mar 30. PMID: 28338370.

31. Gur S, Alzweri L, Yilmaz-Oral D, Kaya-Sezginer E, Abdel-Mageed AB, Dick B, Sikka SC, Volkan Oztekin C, Hellstrom WJG. Testosterone positively regulates functional responses and nitric oxide expression in the isolated human corpus cavernosum. Andrology. 2020 Nov;8(6):1824-1833. doi: 10.1111/andr.12866. Epub 2020 Aug 24. PMID: 32672414.

32. Cabero A. Sofocos, menopausia, óxido nítrico y tratamiento hormonal sustitutivo: "uno para todos y todos para uno" [Hot flashes, menopause, nitric oxide and hormone replacement therapy: "one for all and all for one"]. Med Clin (Barc). 2000 Jan 22;114(2):52-3. Spanish. doi: 10.1016/s0025-7753(00)71187-0. PMID: 10702949.

33. Neeb L, Reuter U. Nitric oxide in migraine. CNS

Neurol Disord Drug Targets. 2007 Aug;6(4):258-64. doi: 10.2174/187152707781387233. PMID: 17691982.

34. Wu G, Meininger CJ, McNeal CJ, Bazer FW, Rhoads JM. Role of L-Arginine in Nitric Oxide Synthesis and Health in Humans. Adv Exp Med Biol. 2021;1332:167-187. doi: 10.1007/978-3-030-74180-8_10. PMID: 34251644.

35. Daiber A, Münzel T, Gori T. Organic nitrates and nitrate tolerance--state of the art and future developments. Adv Pharmacol. 2010;60:177-227. doi: 10.1016/B978-0-12-385061-4.00007-6. PMID: 21081219.

36. Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. Nat Rev Drug Discov. 2021 Sep;20(9):689-709. doi: 10.1038/s41573-021-00233-1. Epub 2021 Jun 30. Erratum in: Nat Rev Drug Discov. 2021 Aug;20(8):652. PMID: 34194012; PMCID: PMC8243062.

37. Tobias MA. Comparison of nitroprusside and nitroglycerine for controlling hypertension during coronary artery surgery. Br J Anaesth. 1981 Aug;53(8):891-7. doi: 10.1093/bja/53.8.891. PMID: 6791673.

38. Roberts TR, Garren MRS, Handa H, Batchinsky AI. Toward an artificial endothelium: Development of blood-compatible surfaces for extracorporeal life support. J Trauma Acute Care Surg. 2020 Aug;89(2S Suppl 2):S59-S68. doi: 10.1097/TA.0000000000002700. PMID: 32251267; PMCID: PMC7398848.

39. O'Connor DM, O'Brien T. Nitric oxide synthase gene therapy: progress and prospects. Expert Opin Biol Ther. 2009 Jul;9(7):867-78. doi: 10.1517/14712590903002047. PMID: 19463074.