ROLE OF NITRIC OXIDE ON CARDIOVASCULAR HEALTH AND DISEASE

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ABSTRACT

Nitric oxide (NO) is a gaseous signalling molecules and cellular messenger that plays key functional role in cardiovascular system. It is continuously synthesized by nitric oxide synthase (NOS) in our body. Nitric oxide synthase (NOS) has three isoforms i.e., neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS). All three isoform of NOS have the same enzymatic function but have minor structural differences which separate them from each other. NO is very much essential for maintenance of blood pressure and other cardiovascular function. Excess or depletion of NO is also responsible for the onset and progression of cardiovascular complication. NO level in cells is also impaired in the absence of cofactors which control NOS activity. It has crucial role in hypertension, atherosclerosis, cardiac ischemia and cardiac contraction. Polymorphism in the three isoforms of NOS causes various cardiovascular diseases i.e., coronary spasm, coronary artery disease, and acute myocardial infarction. Nitrate therapy has been initiated for different kinds of cardiovascular complications especially for coronary artery disease. Nitric oxide blocker especially iNOS inhibitors can be used for chronic inflammation condition like cardiomyopathy and some infectious disease conditions. In this review we discuss about the role of NO in cardiovascular system and its possibility to use as therapies against cardiovascular complications.

Keywords- Nitric Oxide, NOS, Cardiovascular Complication, Polymorphism, Intervention.

I. INTRODUCTION

Nitric oxide (NO) was considered as an important gaseous molecule in biological system when Ferid Murad first found the vasodilatation effect of NO in cardiovascular system. In 1977, he observed that nitroglycerine which released NO in cells could relax the smooth muscle cells [1]. Nowadays NO is regarded as a very important regulator in biological system especially cardiovascular. Its importance is such that more than one lakh papers have been published with NO in the title. Although NO was initially identified in endothelial cells with endothelium-derived relaxing factor, we understand that almost all cells can generate NO and it plays a variety of physiological roles. In general, the roles of NO include the maintenance of vascular tone, acting as a neurotransmitter in both the central and peripheral nervous systems, and mediating cellular defence. Besides these, NO interacts with mitochondrial systems to regulate cell respiration, to increase mitochondrial biogenesis and to augment the generation of reactive oxygen species [2]. All these effects of NO may trigger the mechanisms of cell survival or death. In addition to maintaining a vasodilator tone, NO inhibits platelet aggregation and adhesion, and modulates smooth muscle cell proliferation [3]. NO has been implicated in a

number of cardiovascular disorders. Reduction of endothelial generation of NO is linked to risk of all kinds of cardiovascular disorder. For example, reduced basal NO synthesis or generation leads to vasoconstriction, elevated blood pressure, platelet aggregation and thrombus formation [4]. On the other hand, overproduction of NO leads to enhanced reactive oxygen species which directly or indirectly may cause vascular leakage, and disruption of cell metabolism [5]. Vasodilatation and hypotension are other consequence of excess generation of NO. Appropriate pharmacological or molecular biological manipulation of the generation of NO will definitely prove beneficial in different kinds of cardiovascular disorder. This review will focus on the role of NO on cardiovascular system and its modulation by pharmacological agents.

II. NITRIC OXIDE AND NITRIC OXIDE SYNTHASE (NOS)

Several factors i.e., platelet-derived factors, stress, acetylcholine, and cytokines stimulate the production of NO by nitric oxide synthase (NOS). NO is synthesized by NOS from the terminal guanidine-nitrogen of L-arginine and oxygen. NO and citrulline are formed from L-arginine in presence of NOS (Fig 1). NO gas either diffuses directly to its target, or it is converted to: **(1)** a different oxides of nitrogen, such as NO_2 or N_2O_3; **(2)** an organonitrosyl (E-NO) compound, where E is a sulfur, nitrogen, or carbon containing moiety; or **(3)** a metal-nitrosyl (M-NO) complex. Some of these species are important for delivery of NO and others for long term storage. NO and its derivative can exert its physiological or pathophysiological functions by interaction with redox-active metals bound to or in the vicinity of redox-active ligands or supported by redox-active ligands, such as zinc finger proteins.

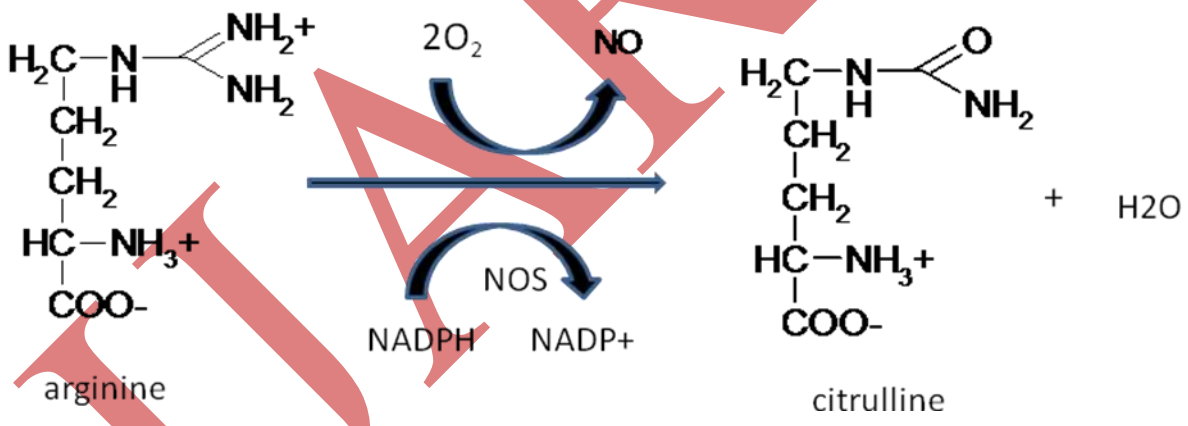

Figure 1. Nitric oxide generation from arginine in presence of NADPH and NOS

Three isoforms of nitric oxide synthase (NOS) enzyme are currently identified i.e., neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS). All three isoforms of NOS have the same mechanism but have minor structural differences which distinguish them from each other (Table 1). One of these major differences is the difference in amino acids at the active site and hydrogen bond with the substrate giving the enzyme its activity. Flinspach et. al. studied selective dipeptide inhibitors to differentiate one isoform from another. A difference in the active sites between eNOS and nNOS allowed these inhibitors to be selective for one isoform over another. It was reported that the selectivity of the inhibitors occurred near the alpha-amino group of the inhibitor. In eNOS this position is occupied by Asn368 whereas Asp597 is accepted in nNOS7. Another structural difference is that iNOS possesses a Asn368 residue

in place of a Ser residue in both eNOS and nNOS. Expression and function of NOS is tissue specific as described on Table 2.

Table 1. Different Types Nitric Oxide Synthase (NOS)

NOS isoform	Gene structure and size	Protein size	References
nNOS (NOS1)	26 exons, 25 introns, 37kbp	1434 aa, 161 kDa	6,7
iNOS (NOS2)	26 exons, 25 introns, 37 kbp	1153 aa, 131 kDa	8,9,10
eNOS (NOS3)	26 exons, 25 introns, 21-22 kbp	1203 aa, 133 kDa	11,12

Table 2. Expression and function of NOS

NOS isoform	Expression in organs	Function
nNOS (NOS1)	Brain, skeletal muscle, pancreas, heart.	Neurotransmission
iNOS (NOS2)	Macrophages, smooth muscle, heart, liver.	Inflammation, septic shock, Cytotoxicity,
eNOS (NOS3)	Endothelium, brain, skin.	Vasodilation and leukocyte adhesion.

III. NITRIC OXIDE AND CARDIOVASCULAR FUNCTION

Nitric oxide (NO) is an important signaling molecule in the cardiovascular system. NO serves many important biological functions in cardiovascular physiology and acts as endothelium-derived relaxing factor (EDRF). NO inhibits platelet aggregation, leukocyte endothelium adhesion, vascular smooth muscle proliferation and thus maintains vascular integrity. NO produced in cardiac muscle regulates cardiac contractility. Diminished NO bioavailability in blood vessels is called endothelial dysfunction, leading to increase susceptibility to atherosclerotic disease. Diseases like hypertension, diabetes mellitus, atherosclerosis, hypercholesterolemia, congestive heart failure, thrombosis and stroke are associated with NO deficiency and abnormalities in NO signalling [13].

3.1 Nitric Oxide And Hypertension

Hypertension is an important risk factor for cardiovascular disease. Reduction of elevated blood pressure reduces the risk of cardiovascular events. There are several interacting homeostatic regulators of blood pressure ie, renin–angiotensin system, the autonomic nervous system, and local mediators like EDRF. Nitric oxide (NO) bioavailability is directly linked to endothelial dysfunction and plays a major role in regulating blood pressure. Impaired NO bioactivity is associated with hypertension. Elevated blood pressure level is seen in case of the disruption of the gene encoding endothelial NO synthase. This suggests that genetic component draws the link between impaired NO bioactivity and hypertension. NOS inhibitors cause a rise in blood pressure in many

species, including mice, rats, guinea pigs, rabbits, dogs. eNOS knockout mice also showed 30% higher blood pressure than that of wild type mice. Patients with hypertension have a blunted arterial vasodilatory response after infusion of endothelium-dependent vasodilators. The major mechanism of systolic hypertension is arterial stiffness which occurs due to impairment of NO bioactivity. Thus, eNOS plays an important role and key player in regulation of blood pressure [14].

3.2 Nitric Oxide And Atherosclerosis

Atherosclerosis is a process in which biochemical, cellular, and physiological forces in the vessel wall cause vascular injury and lead to endothelial dysfunction, cellular proliferation, recruitment of inflammatory cells, and accumulation of oxidized LDL. Neointima is formed in response to blood vessel injury. NO has protective role in vessel injury by suppressing smooth muscle proliferation. By inhibition of platelet aggregation and adhesion, and inhibition of leukocyte activation and adhesion, NO suppresses the development of atherosclerotic plaques. Deficiency in vascular NO enhances the risk of atherosclerosis. eNOS knockout mice show significantly greater neointima formation after cuff injury than wild type mice. Thus, deficiency in the amount of available NO in the vessel wall increases neointimal formation in response to vascular injury [15].

3.3 Nitric Oxide And Ischaemic Preconditioning

Ischaemic preconditioning is a protective response against subsequent, more severe ischaemia. The potential protective mechanisms include alterations in cell death, gene expression, heat shock proteins, lipid peroxidation, inflammation, and mitochondrial metabolism. NO has important protective roles on heart not only through increasing blood flow but also by directly enhancing cardioprotection. Myocardial ischaemia reperfusion injury and cardiac ischaemic preconditioning depend mainly on iNOS. Molecular mechanisms of iNOS protection involve electron transport or the mitochondrial permeability transition pore [16].

3.4 Cardiac Contractility

NO plays a significant role in regulating cardiac contractility. Endothelial cells and cardiac myocytes produce nitric oxide in the heart. Endothelial cells are very rich in eNOS and line the vasculature and endocardium. NO is essential for cardiac excitation-contraction coupling. Cardiac myocytes express both eNOS and nNOS. In cardiac myocytes, eNOS is localized to the sarcolemmal caveolae and interacts with caveolin-3. On the other hand, nNOS is localized to the sarcoplasmic reticulum and is associated with the ryanodine receptor. Thus eNOS and nNOS play distinct roles in cardiac function by resideing in different subcellular locations [17].

IV. IMPAIRMENT OF NO AVAILABILITY

NO is very much important to the pathophysiology of vascular disease and endothelial dysfunction. Endothelial function is impaired before structural changes such as intimal hyperplasia, lipid deposition or vascular injury. The early endothelial dysfunction is characterized by diminished endothelial NO production. There are several mechanisms that can induce endothelial dysfunction. These are reduced eNOS expression levels, reduced eNOS enzymatic activity, and rapid removal of NO from cell and blood. 1) Changes in eNOS mRNA and protein expression directly affect eNOS activity. 2) The availability of L-arginine, the substrate for NO production, may limit the NO production in tissues. 3) Asymmetric dimethyl arginine reduces endothelial NO production by acting as competitive inhibitor with L-arginine [18]. 4) eNOS requires FAD, FMN, NADPH, and BH4 as

cofactors to function properly. In the absence of these cofactors electron transport through eNOS can become 'uncoupled,' resulting in generation of superoxide anion [19]. 5) eNOS requires dimerization and proper intracellular localization to caveolae, through interactions with caveolin and hsp90. Any alteration of dimerization and intracellular localisation may affect eNOS function.

V. NOS POLYMORPHISM AND CARDIOVASCULAR DISORDER

Several polymorphisms have been identified in the eNOS gene. Among them, a common variant located in exon 7 (G984T) of the eNOS gene that modifies its coding sequence (Glu298Asp) has been linked by several investigators to the risk of cardiovascular diseases such as for coronary spasm, coronary artery disease (CAD), and acute myocardial infarction [20]. One study reported that the Glu298Asp polymorphism is associated with the occurrence and severity of CAD in the Italian population [21]. However, for atherosclerosis the relationship between the Asp variant and the risk was not established. Glu298Asp polymorphism in the eNOS gene did not increase the susceptibility to coronary and carotid arteries disease in patients [20]. Both Glu298Asp and T786C (promoter region) polymorphisms of the eNOS gene are associated with the severity of angiographically defined coronary artery disease in the Italian population. Individuals carrying both eNOS variants might have a higher risk of developing coronary artery disease compared to those individuals carrying any single polymorphoism [22]. T-786C a polymorphism in promoter region of eNOS gene affects cerebral circulation in smokers [23]. Meta analysis was conducted to identify the association between eNOS gene G894T (Glu298Asp) polymorphism and the risk of ischemic stroke [24]. G894T(Glu298Asp) was identified as a new genetic risk factor for acute myocardial infarction (AMI). Data confirmed that homozygosity for the Glu298Asp polymorphism of the eNOS gene may be involved in predisposition to AMI [25]. However, all of the above polymorphism can explain only a small part of genetic susceptibility to cardiovascular diseases. Further studies are needed to characterize the molecular mechanisms by which eNOS is involved in susceptibility to cardiovascular disorders.

VI. NO, AND ITS MODULATION

Nitric oxide had vasodilator and platelet inhibitory actions. NO was found to inhibit vascular smooth muscle proliferation and regulate interactions between leucocytes and the blood vessel wall. These established NO as a homeostatic regulator in the vasculature, the absence of which causes number of disease conditions and pathological states such as hypertension and vasospasm [26, 27]. Previously, all these pathological conditions were collectively called endothelial dysfunction. Endothelial dysfunction is characterised by the reduction in endothelial NO. Endothelial dysfunction is detected prior to any other evidence of cardiovascular disease like essential hypertension and atherosclerosis. Lack of NO enhances endothelial dysfunction and on the other hand, excess of NO may generate reactive oxygen species (ROS) and cause inflammation. Inflammatory stimuli such as endotoxin lipopolysaccharide and cytokines induce NO production through iNOS in many cells and tissues. This enzyme was identified originally in macrophages and contributes to the inflammatory and cytotoxic actions of these cells. It is inhibited by anti-inflammatory glucocorticoids. Profound vasodilatation in septic shock was mediated through excess NO produced by iNOS in the vasculature [26]. Similarly endotoxin also induces iNOS in the myocardium [28] and responsible for cardiac dysfunction and damage. Modulation of NO is used to control different kind of pathological conditions in human. Vasodilatory effect of NO is mostly utilised in many

disease conditions. Nitrates are being used to treat angina pectoris since more than a century. Nitrates release nitric oxide, which causes vasodilation of coronary arteries. Some nitrates like nitroprusside, automatically decompose to nitric oxide. Some others nitrates like nitroglycerin and isosorbide dinitrate, are metabolized by cells into nitric oxide. These nitrates are used as vasodilators to treat hypertension, angina and hypertensive crises. Nitric oxide inhalation is used as a bronchodilator to treat asthma and chronic obstructive pulmonary disease by directly relaxing constricted bronchial smooth muscle. Inhaled nitric oxide reduced pulmonary vascular resistance and thus reduced pulmonary hypertension by dilating pulmonary arteries [29]. Nitrates are also used for the treatment of adult respiratory distress syndrome by reducing pulmonary artery pressure. Nitric oxide has shown to reduce the size of myocardial infarctions in some animals [30]. Another kind of NO therapy is to reduce excess NO production by NOS inhibition. Nitric oxide synthase inhibitors successfully treat the hypotension in sepsis. Nitric oxide synthase inhibitors can reduce the infarction size of strokes in animal studies [31,32,33]. However, the use of nitric oxide synthase inhibitors is limited as nitric oxide is involved in many different physiologic functions. The most commonly used nitric oxide synthase inhibitors are compounds related to the nitric oxide synthase substrate arginine, such as N-mono-methyl-arginine or N-nitro-arginine methyl ester. However, these compounds are nonselective blockers of nitric oxide synthase. Specific nitric oxide synthase inhibitors is required to inhibit specific NOS depending on the induction in a disease state.

VII. CONCLUSION

Extensive research on nitric oxide has shown that NO plays an important role in various physiological function. However the molecular targets of nitric oxide need to be more precisely defined, perhaps the mechanism by which NO regulates different important enzymes through nitrosylation. Research on the regulation of nitric oxide synthase should lead to a better understanding of its role in the pathogenesis of various diseases. There is a need of nitric oxide synthase inhibitors to block specific isoforms of nitric oxide and stable compounds that release NO slowly. Finding novel molecules that regulate NO and normalise its function in different disease states might revolutionise the NO therapy.

REFERENCE

[1] S. Mattapally, and S.K Banerjee, Nitric oxide: redox balance, protein modification and therapeutic potential in cardiovascular system, IIOABJ, 2(6), 2011, 29–38.

[2] W.K. Alderton, C.E. Cooper, and R.G. Knowles, Review article Nitric oxide synthases : structure, function and inhibition. Biochem. J., 357, 2001, 593-615.

[3] S.C. Tyagi, and M.R. Hayden, Role of Nitric Oxide in Matrix Remodeling in Diabetes and Heart Failure, Heart Failure Reviews, 8, 2003, 23–28.

[4] K. M. Naseem, The role of nitric oxide in cardiovascular diseases, Molecular Aspects of Medicine, 26(1–2), 2005, 33–65.

[5] S. Moncada, and E.A Higgs. The discovery of nitric oxide and its role in vascular biology, British Journal of Pharmacology, 147, 2006, S193–S201.

[6] M. Nakane, , H. H. Schmidt, J. S. Pollock, U. Forstermann, and F. Murad, Cloned human brain nitric oxide synthase is highly expressed in skeletal muscle, FEBS Lett, 1993, 316, 175-180.

[7] A. V. Hall, H. Antoniou, Y. Wang, A. H. Cheung, A. M. Arbus, S. L. Olson, W. C. Lu, C. L. Kau, and P. A. Marsden, Structural organization of the human neuronal nitric oxide synthase gene (NOS1), J. Biol. Chem, 269, 1994, 33082-33090.

[8] D. A. Geller, C. J. Lowenstein, R. A. Shapiro, A. K. Nussler, M. Di Silvio, S. C. Wang, D. K. Nakayama, R. L. Simmons, S. H. Snyder, and T. R. Billiar, Molecular cloning and expression of inducible nitric oxide synthase from human hepatocytes. Proc. Natl. Acad. Sci. U.S.A. 90, 1993, 3491-3495.

[9] P. A. Sherman, V. E. Laubach, B. R. Reep, and E. R. Wood, Puri®cation and cDNA sequence of an inducible nitric oxide synthase from a human tumor cell line, Biochemistry, 32, 1993, 11600-11605.

[10] I. G. Charles, R. M. Palmer, M. S. Hickery, M. T. Bayliss, A. P. Chubb, V. S. Hall, D. W. Moss, and S. Moncada, Cloning, characterization and expression of a cDNA encoding an inducible nitric oxide synthase from the human chondrocyte, Proc. Natl. Acad. Sci. U.S.A, 90, 1993, 11419-11423.

[11] S. P. Janssens, A. Shimouchi, T. Quertermous, D. B. Bloch and K. D. Bloch, Cloning and expression of a cDNA encoding human endothelium-derived relaxing factor/nitric oxide synthase, J. Biol. Chem, 267, 1992, 14519-14522.

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

[13] K. M. Naseem, The role of nitric oxide in cardiovascular diseases, Molecular Aspects of Medicine, 26, 2005, 33–65.

[14] J.A. Panza, P.R. Casino, C.M. Kilcoyne, and A.A. Quyyumi, Role of endothelium- derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension, Circulation, 87, 1993, 1468–1474.

[15] V.W.T. Liu and P.L. Huang, Cardiovascular roles of nitric oxide: A review of insights from nitric oxide synthase gene disrupted mice, Cardiovascular Research, 77, 2008, 19–29.

[16] Y. Guo, A.B. Stein, W.J. Wu, X. Zhu, W. Tan, Q. Li, and R. Bolli, Late preconditioning induced by NO donors, adenosine A1 receptor agonists, and fdeltag1-opioid receptor agonists is mediated by iNOS, Am J Physiol Heart Circ Physiol, 289, 2005, H2251–H2257.

[17] P.B. Massion, O. Feron, C. Dessy and J.L. Balligand, Nitric Oxide and Cardiac Function: Ten Years After, and Continuing, Circ Res., 93, 2003, 388-398.

[18] S.M. Bode-Boger, R.H. Boger, S. Kienke, W. Junker, and J.C. Frolich. Elevated L-arginine/dimethylarginine ratio contributes to enhanced systemic NO production by dietary L-arginine in hypercholesterolemic rabbits, Biochem Biophys Res Commun, 219,1996, 598–603.

[19] F. Cosentino, S. Patton, L.V. d'Uscio, E.R. Werner, G. Werner-Felmayer, P. Moreau, T. Malinski, and T.F. Lüscher, Tetrahydrobiopterin alters superoxide and nitric oxide release in prehypertensive rats, J Clin Invest, 101, 199, 1530–1537.

[20] H. Younan, G.A. Razek, K. Elkhashab, H. Abdelrasol, and M. Saad, Relationship of endothelial nitric oxide synthase gene polymorphism with atherosclerotic coronary and carotid arterial disease in Egyptian population, The Egyptian Heart Journal, 2014, (in press).

[21] A.D. Hingorani, C.F. Liang, J. Fatibene, A. Lyon, S. Monteith, A. Parsons, S. Haydock, R.V. Hopper, N.G. Stephens, K.M. O'Shaughnessy, and M.J. Brown. A common variant of the endothelial nitric oxide synthase

(Glu2983Asp) is a major risk factor for coronary artery disease in the UK, Circulation, 100, 1999, 1515–1520.

[22] M.G. Colombo, U. Paradossi, M.G. Andreassi, N. Botto, S. Manfredi, S. Masetti, A. Biagini, and A. Clerico, Endothelial Nitric Oxide Synthase Gene Polymorphisms and Risk of Coronary Artery Disease, Clinical Chemistry, 49(3), 2003, 389–395.

[23] S. Nasreen, T. Nabika, H. Shibata, H. Moriyama, K. Yamashita, J. Masuda, and S. Kobayashi, T-786C Polymorphism in Endothelial NO Synthase Gene Affects Cerebral Circulation in Smokers Possible Gene-Environmental Interaction, Arterioscler Thromb Vasc Biol, 22, 2002, 605-610.

[24] M. Wang, X. Jiang, W. Wu, and D. Zhang, Association of G894T polymorphism in endothelial nitric oxide synthase gene with the risk of ischemic stroke: A meta-analysis, Biomedical Reports, 1, 2013, 144-150.

[25] K. Hibi, T. Ishigami, K. Tamura, S. Mizushima, N. Nyui, T. Fujita, H. Ochiai, M. Kosuge, Y. Watanabe, Y. Yoshii, M. Kihara, K. Kimura, M. Ishii, and S. Umemura, Endothelial Nitric Oxide Synthase Gene Polymorphism and Acute Myocardial Infarction, Hypertension, 32, 1998, 521-526.

[26] S. Moncada, and E.A. Higgs (2000). Nitric oxide in cardiovascular function and disease. In: Atherosclerosis XII, Proceedings of the XIIth International Symposium on Atherosclerosis, Stockholm, The Netherlands: Elsevier eds, 81–89.

[27] D.D. Rees, E.A. Higgs, and S. Moncada (2000). Nitric oxide and the vessel wall. In: Hemostasis and Thrombosis, eds. Colman, R.W., Hirsch, J., Marder, V.J., Clowes, A.W. & George, N.J., pp. 673–682. Lippincott Williams & Wilkins: Philadelphia.

[28] R. Schulz, E. Nava, and S. Moncada, Induction and potential biological relevance of a Ca2+-independent nitric oxide synthase in the myocardium, Br. J Pharmacol., 105, 1992, 575–580.

[29] J.D. Roberts, D.M. Polaner, P. Lang, and W.M. Zapol, Inhaled nitric oxide in persistent pulmonary hypertension of the newborn, Lancet, 340, 1992, 818-819.

[30] G. Johnson, P.S. Tsao, and A.M. Lefer, Cardioprotective effects of authentic nitric oxide in myocardial ischemia with reperfusion, Crit Care Med, 19, 1991, 244-252.

[31] T.M. Dawson, V.L. Dawson, and S.H. Snyder, A novel neuronal messenger molecule in brain: the free radical, nitric oxide, Ann Neurol, 32, 1992, 297-311.

[32] J.P. Nowicki, D. Duval, H. Poignet, and B. Scatton, Nitric oxide mediates neuronal death after focal cerebral ischemia in the mouse, Eur J Pharmacol, 204,1991, 339-340.

[33] D.A. Dawson, K. Kusumoto, D.I. Graham, J. McCulloch, and I.M. Macrae, Inhibition of nitric oxide synthesis does not reduce infarct volume in a rat model of focal cerebral ischaemia, Neurosci Lett 142, 1992, 151-154.