Effects of Chronic nitric Oxide inhibition on Cardiac and hemodynamic Functions in rats

Thesis

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Abstract

Key Words : nitric oxide , hypertention , free radicals .

Nitric oxide is an important messenger molecule that have a vital role as a vasodilator and blood pressure regulator .

Nitric oxide regulates blood pressure by a signalling mechanism involving cyclic GMP .

However , nitric oxide works by another mechanism in protecting the endothelium . by acting as a free radical scavenger .

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List of Abbreviations

ADMA : Asymmetric Dimethyl arginine .

ANP : Atrial natriuretic peptide .

ATP : Adenosine triphosphate .

BH4 : Tetrahydrobiopetrin .

BW : Body weight .

cAMP : Cyclic adenosine monophosphate .

CAPON : Carboxyl-terminal PDZ ligand of NOS .

CFTR : Cystic fibrosis transport protein regulator .

CHF : congestive heart failure .

cGK : cyclic GMP dependent kinase .

cGMP : Cyclic guanosine monophosphate .

CNG : cyclic nucleotide-gated channels .

CW : Cardiac weight .

DAG : diacyl glycerol .

DDAH : dimethyl diamino hydrolase

eNOS : Endothelial nitric oxide synthase .

FAD : Flavin adenine dinucleotide .

FMD : Flow mediated dilatation .

FMN : Flavin adenin mononucleotide .

GC : Guanulate cyclase .

GTP : Guanosine triphosphate .

H2O2 : Hydrogen peroxide .

LDL : low density lipoprotein .

L – NAME : Nitro – L – arginine methyl ester .

LVW : left ventricular weight .

iNOS : Inducible nitric oxide synthase .

JAK : Janus kinase .

MLC : Myosin light chain .

MPO : Myeloperoxidase .

NFK – B : Nuclear factor Kappa B .

NMDA : N methyl D aspartate .

nNOS : Neuronal nitric oxide synthase .

NO+ : nitrosonium

NO− nitroxyl anion

NO : nitric oxide .

NOS : Nitric oxide synthase .

NTG : nitroglycerin .

O2 - : superoxide anion .

OH - : hydroxyl radical .

ONOO - : Peroxinitrite .

PDE : Phosphodiesterase .

PET : Positron emmission tomography .

PIP2 : phosphatidyl inositol biphosphate .

PKA : Protein kinase A .

PKB : Protein kinase B .

PKC : Protein kinase C .

PLB : Phospholamban .

PLC : Phospholipase C .

PPAR-g : peroxisome proliferator-activated receptor-g [Thiazolidinediones] .

(PSD-95) : post-synaptic density protein .

RNS : reactive nitrogen species.

ROS : Reactive oxygen species .

SAM : S Adenosyl methionine .

SNP : Sodium nitropruside .

SREBP : Sterol regulatory element binding protein .

STAT : signal transducers and activators of transcription .

TCP : Tail cuff pressure .

VIP : Vasoactive intestinal peptide .

VSM : Vascular smooth muscles .

INTRODUCTION AND AIM OF THE WORK

Nitric oxide is an important messenger molecule in mammals and other animals. It can be toxic or beneficial, depending on the amount and where in the body it is released. Initial research into the chemistry of nitric oxide (NO) was motivated by its production in car engines, which results in photochemical smog and acid rain. In the late 1980s, researchers in immunology, cardiovascular pharmacology, neurobiology, and toxicology discovered that nitric oxide is a crucial physiological messenger molecule **[Murad , 2004]**

On October 12th 1998, the Nobel Assembly awarded the Nobel Prize in Medicine and Physiology to scientists Robert Furchgott, Louis Ignarro, and Ferid Murad for their discoveries concerning nitric oxide (NO) as a signaling molecule in the cardiovascular system. In contrast with the short research history of the enzymatic synthesis of NO, the introduction of nitrate-containing compounds for medicinal purposes marked its 150th anniversary in 1997 **[Murad , 2004].**

Glyceryl trinitrate (nitroglycerin,NTG) is the first compound of this category. Alfred Nobel (the founder of Nobel Prize) himself had suffered from angina pectoris and was prescribed nitroglycerin for his chest pain. Almost a century later, research in the NO field has dramatically extended and the role of NO in physiology and pathology has been extensively studied **[Murad , 2004] .**

The steady-state concentration and the biological effects of NO are critically determined not only by its rate of formation, but also by its rate of decomposition. Bio transformation of NO and its related N-oxides occurs via different metabolic routes within the body and presents another attractive field for our research as well as for the venture of drug discovery **[Bian et al , 2003]**

In 1990 , Furchgott and Zawadiski discovered that the ability of acetyl choline to relax vascular smooth muscles does not involve direct actions through cholinergic receptors as what was thought before , instead , actions of acetylcholine discovered to involve a signaling process that requires the endothelium to elaborate a vasoactive substance that would enter the smooth muscle to relax it causing subsequent vasodilatation , which was discovered to be through nitric oxide .

Nitric oxide is now thought to play a role in blood pressure regulation , control of blood clotting, immune defense, digestion, the senses of sight and smell , and possibly learning and memory. Also playing a prominent role in skeletal muscle and neural transmission **[Bredt et al , 1990] ,** Nitric oxide may also participate in disease processes such as diabetes, stroke, hypertension, impotence, septic shock, long-term depression and potentiation , and in regulating heart structure and function **[Aldestron et al , 2001] .**

Aim of the work

This study was done to :

1- Investigate the cardiac and hemodynamic changes as a result of chronic nitric oxide inhibition .

2- Determine the possible signalling pathway downstream that is responsible for such changes .

3- Study wheather nitric oxide as a free radical is benefetial or injurious in the normal physiological dose and state .

Physical and chemical properties of nitric oxide

Most cellular messengers are large, unreactive biomolecules that make specific contacts with their targets. In contrast, nitric oxide is a small molecule that contains a free radical—that is, an unpaired electron—making it very reactive. Being a gas , Nitric oxide can freely diffuse through aqueous solutions or membranes, reacting rapidly with metal centers in cellular proteins and with reactive groups in other cellular molecules. It is the first gas to be known to work as biological messenger in mammals . NO does not dimerize or dismutate , being a gas , its half life is short , less than 10 seconds **(Stamler et al , 1992) .**

Free radicals:

Rowe (1992) reported that free radicals are highly reactive atoms or molecules bearing one or more unpaired electron, which can cause random damage to structural proteins, enzymes and deoxyribonucleic acid (DNA).

Sue Gillbert (1999) found out that free radicals are the byproduct of a normal event which is the burning of oxygen by the body cells to produce energy. These byproduct molecules are missing an electron, and will attack any nearby molecule to get it. If they take this electron from important components in the body like DNA, proteins or fat, they will damage these components and lead to health problem.

According to **Cheesman et al , (1993)** , this means that these free radicals are trying to gain stability by capturing the needed electron, therefore they react quickly with their surroundings, and hence they will attack the nearest stable molecule to steal its electron. When the attacked molecule loses its electron it will become a free radical itself beginning a chain reaction resulting in disruption of living cells. There are many types of free radicals: Superoxide radical (O_2)-, Hydrogen peroxide (H_2O2), Hydroxyl radical (OH"), Singlet Oxygen ($^1O2$), Nitric oxide (NO) and Peroxynitrite (ONOO).

Synthesis of nitric oxide

Usually , chemical transmission occurs in human being through endogenous chemical mediators , which are synthesized in sufficient amounts and stored in vesicles , and then released on demand . Usually there is a large storage pool so that the released amount represents small percentage of the total store . However , being a gas , nitric oxide cannot be synthesized in large amounts or stored in vesicles as usual , alternatively , it is synthesized and work by readily permeating the biological membranes , binding with guanylyl cyclase , an " enzyme receptor " , and as there is not a large storage pool , so this signaling is regulated specifically at the level of synthesis **(Stojanovic et al , 2004) .**

Nitric oxide is synthesized through the action of a group of enzymes known collectively as nitric oxide synthase [NOS] , which is one of the most known enzymes to be highly regulated at the level of synthesis **(Aldestron et al , 2001)**

Synthesis , activators and inhibitors of nitric oxide synthesis can be shown in the following diagram : **(Indian journal of physiology , 2005)**