Biological Effects of Nitric Oxide and its Role in Cell Signaling

What is Nitric Oxide?

- First described in 1979 as a potent relaxant of peripheral vascular smooth muscle.
- Used by the body as a signaling molecule.
- Serves different functions depending on body system. i.e. neurotransmitter, vasodilator, bactericide.
- Environmental Pollutant
- First gas known to act as a biological messenger

Background Information

- Prior to 1990: An air pollutant
- Named **"Molecule of the Year"** by Science magazine in 1992

- **Robert Furchgott, Louis J Ignore, Ferid Murad:**

  Nobel Prize 1998 "Discovery of the role of nitric oxide as a signal molecule in the cardiovascular system. ""

- Properties of NO:
  - Small water and lipid soluble gas
  - Gaseous free radical

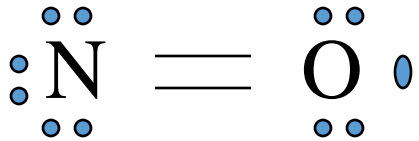
- Three interchangeable forms:

  NO: Nitric Oxide

  NO^+: Nitrosonium cation

  NO^- : Nitroxyl Radical

The structure and nature of Nitric Oxide



- Nitric oxide is a diatomic free radical consisting of one atom of nitrogen and one atom of oxygen
- Lipid soluble and very small for easy passage between cell membranes
- Short lived, usually degraded or reacted within a few seconds
- The natural form is a gas

- ***NO: Unique messenger and play important role in following functions:***

  - Neuronal signaling
  - Penile erection
  - Cardiovascular homeostasis
  - Decompensation in atherogenesis

NOBEL PRIZE

- Dr. Robert Farchgott, an 82-year-old pharmacist at the University of New York, studying the effects of medication on blood vessels, first noticed that the same drugs in some cases cause enlargement, and in others - the narrowing of the same vessels.
- The scientist was interested in whether the opposite results can depend on the condition of the inner surface (endothelium) of the cells inside the blood vessels.

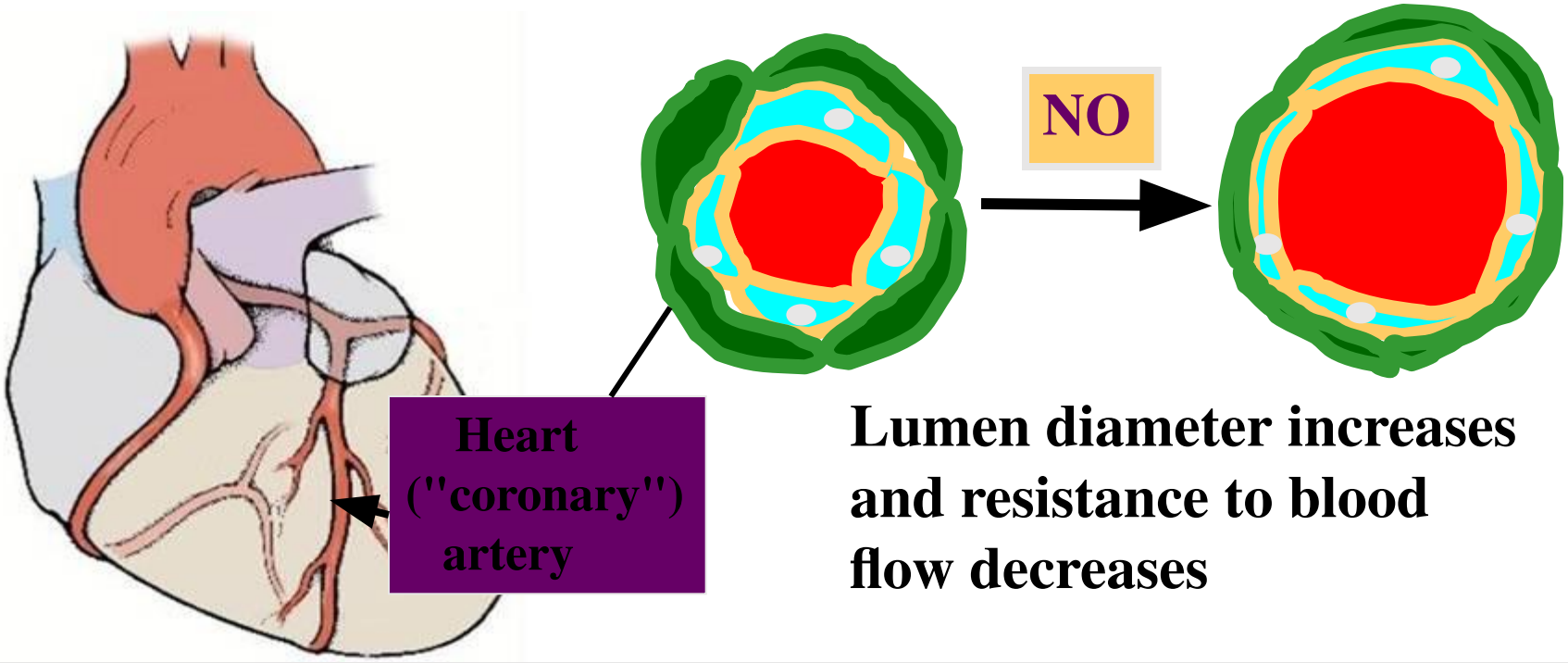
NOBEL PRIZE

- In 1980, in a simple experiment with acetilcholine, he showed that this substance dilates the blood vessels in those cases when the wall of the vessels is not damaged.
- R. Farchgotta concluded **that intact endothelial cells produce an unknown signal hitherto relaxing the smooth musculature of the vessels.**
- This scientist called the molecule **EDRF**, which meant "endothelium-receiving-distributing factor." In search of an unknown signal molecule,
- In search of an unknown signal molecule, independently of R. Farchgott, Dr. Louis Ignarro, a 57-year-old scientist from the University of California at Los Angeles (UCLA), took part. In search of the chemical nature of EDRF, L. Ignarro conducted a brilliant series of studies and in 1986 came to the conclusion that **EDRF is identical to nitric oxide.**

NOBEL PRIZE

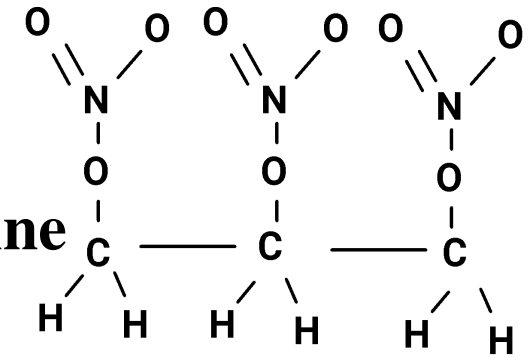
- 62-year-old pharmacologist Ferid Murad from the University of Texas Medical School in Houston analyzed the pharmacological effect of giving nitroglycerin and other related vasodilators.
- In 1977, he established that these substances release nitric oxide, which expands the smooth muscle of cells.
- The idea that gas can regulate the most important cellular functions, seized him, but at that time he did not have sufficient experimental justifications to confirm this idea.

Nitroglycerine was used for many years to treat "angina" (chest pain) due to reduced blood flow in heart arteries without <u>any</u> knowledge of mechanism



Lumen diameter increases and resistance to blood flow decreases

Nitro glycerine



We now know nitroglycerine does not act directly but is degraded to NO

N-O

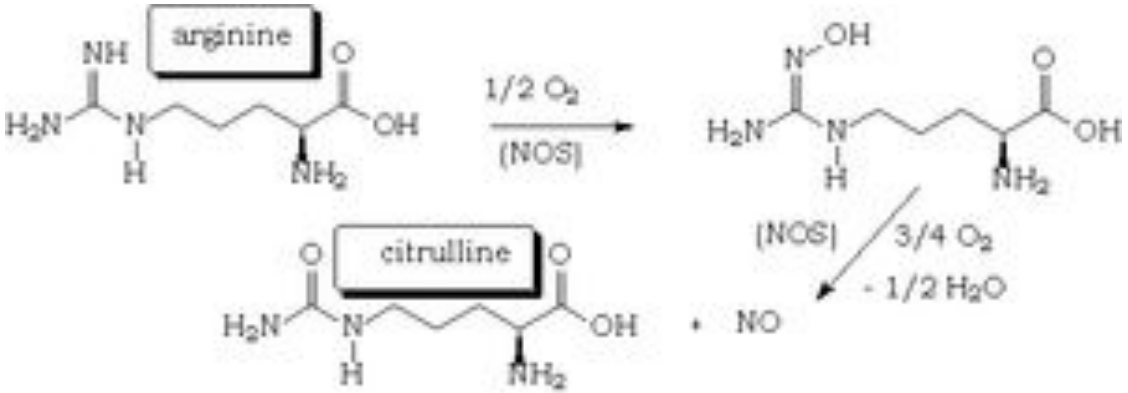
NO functions

- This was the first discovery that gas can act as a molecule signal in the body.
- It turned out that nitric oxide protects the heart, stimulates the brain, kills bacteria, etc.
- Further results confirmed that nitric oxide is a signal molecule, primarily for the cardiovascular system, as well as for a number of other functions, for example, as a signal molecule in Nervous system.

Induction of biosynthesis

Various factors secreted by platelets, in particular, certain prostaglandins, mechanical damage to the vascular endothelium, hypoxia, the impact of such endogenous vasodilator substances, such as acetylcholine, adenosine, histamine, a number of cytokines, stimulation of β-adrenoceptors or 5-HT1A-receptors in the walls of blood vessels lead to increased activity of endothelial nitric oxide synthases (eNOS) and increased biosynthesis of nitric oxide (II). Thus, the vasodilatory action of acetylcholine, histamine, adenosine, prostaglandins is implemented partly via the increase in NO biosynthesis (although this is not the only mechanism of vasodilator action). In contrast, stimulation of α-adrenergic or 5-HT2-receptors of vascular walls leads to a decrease in NO biosynthesis, which is one of the mechanisms caused by catecholamines and serotonin vasoconstriction, although, again, not the only one.

Endothelial synthase of nitric oxide synthesis of nitric oxide (II) from the terminal guanidine nitrogen of L-arginine, as a by-product of the reaction is formed L-citrulline. The formation of nitrogen oxide (II) syntase endothelial nitric oxide requires the participation of tetrahydrobiopterin, NADP, calcium and calmodulin and other cofactors

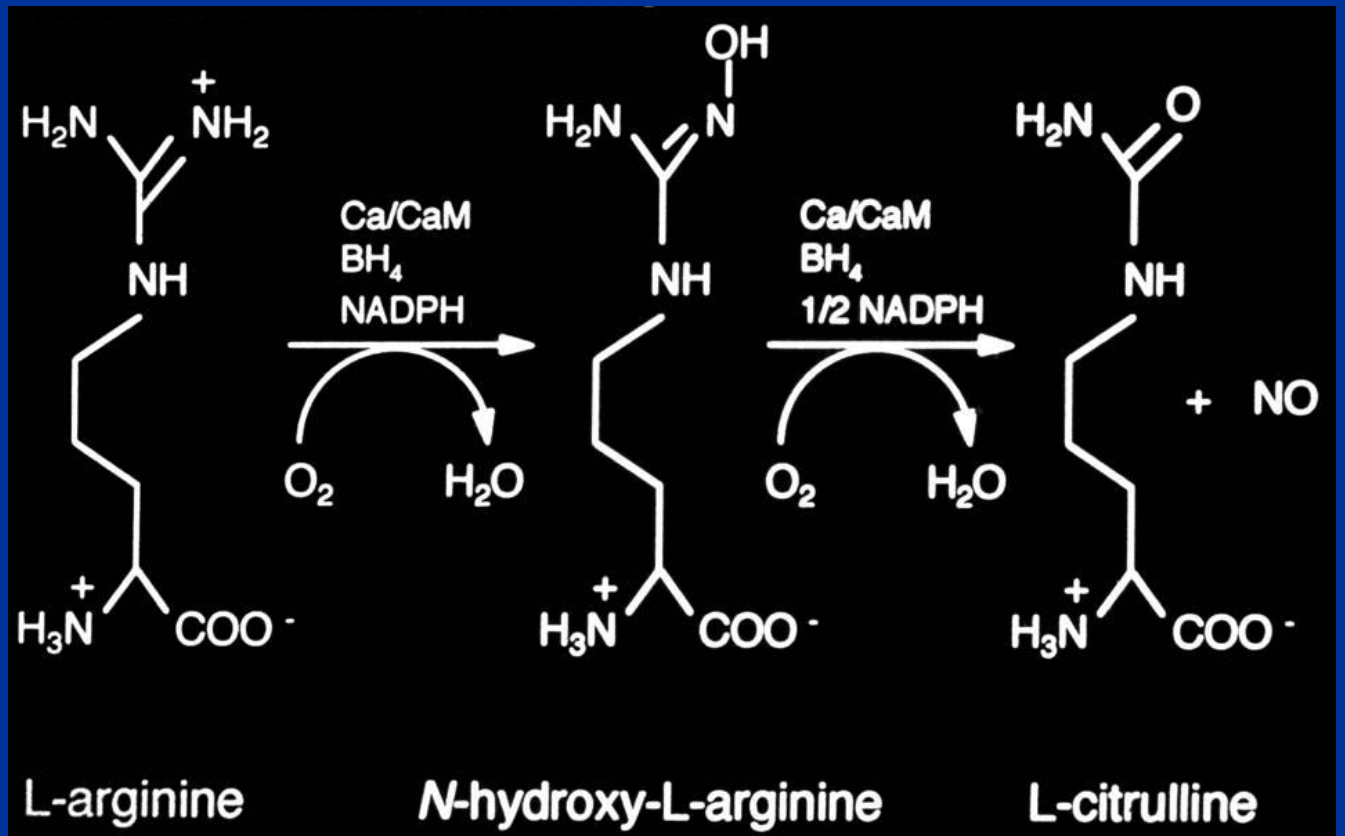


Intracellular signaling cascade

- Nitric oxide (II), a highly reactive free radical, diffuses through the cell membrane of smooth muscle cells of blood vessels and interacts with the heme prosthetic group of soluble called guanylate cyclase, nitrospirae it and the resulting disconnection of the iron heme with a proximal leucine and configuration change of the enzyme, leading to its activation. Guanylate cyclase activation leads to increased formation in the secondary cell mediator cyclic GMP (cGMP) — (3',5'-guanosine-monophosphate) from GTP (guanosine triphosphate). In addition, nitric oxide (II) is also nitrosonium group other important heme iron enzymes, in particular cytochromes and cytochromoxidase, which leads to inhibition of their activity, slowing the rate of oxidative metabolism in mitochondria and reduce oxygen consumption of the smooth muscle cell (which is important in hypoxia conditions).

Synthesis of NO

- NO is synthesized by nitric oxide synthase (NOS) which oxidizes a guanidine nitrogen of L-arginine releasing nitric oxide in the form of a free radical and citrulline.



Nitric Oxide Synthase Isoforms

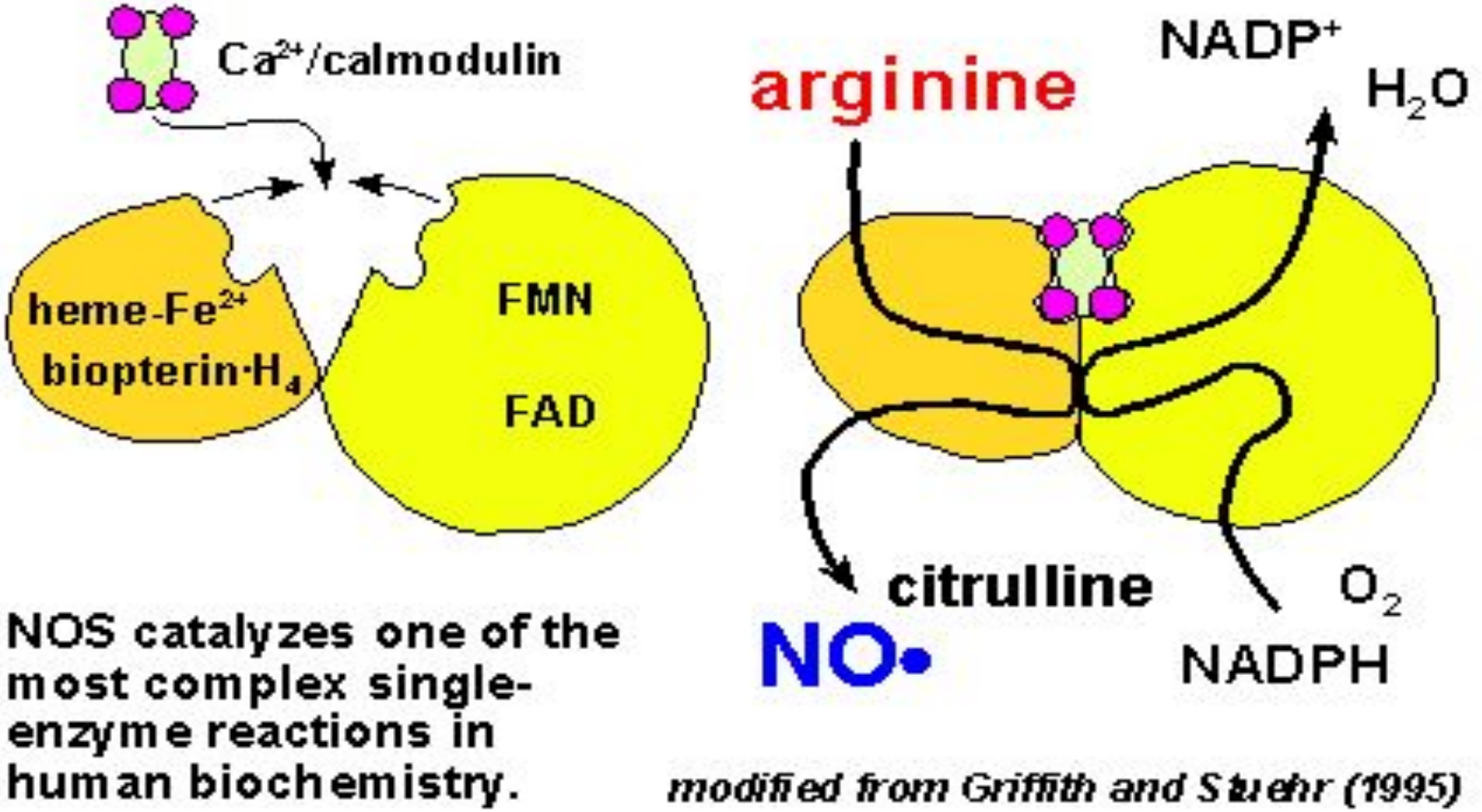
NOS-1 (155kD)	neuronal, brain, Type I-NOS; central and peripheral neurons, NANC neurons, islets, endometrium, skeletal muscle, etc.
NOS-2 (125kD)	inducible, Type II-NOS; macrophage, liver, smooth muscle, endothelium, heart, etc; effects of LPS, cytokines and glucocorticoids
NOS-3 (135kD)	endothelial, Type III-NOS; endothelium, brain, heart, etc.; acylation, phosphorylation

Types of NOS

- NOS I
  - Central and peripheral neuronal cells
  - Ca+2 dependent, used for neuronal communication
- NOS II
  - Most nucleated cells, particularly macrophages
  - Independent of intracellular Ca+2
  - Inducible in presence of inflammatory cytokines
- NOS III
  - Vascular endothelial cells
  - Ca+2 dependent
  - Vascular regulation

Nitric Oxide Synthase (NOS)

substrates, cofactors, and overall reaction



NOS catalyzes one of the most complex single-enzyme reactions in human biochemistry.

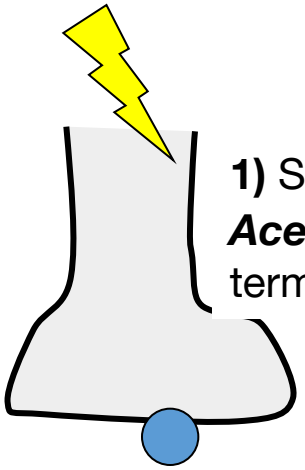
modified from Griffith and Stuehr (1995)

Http://www.kumc.edu/research/medicine/biochemistry/bioc800/sig02-06.htm

Activation of NOS

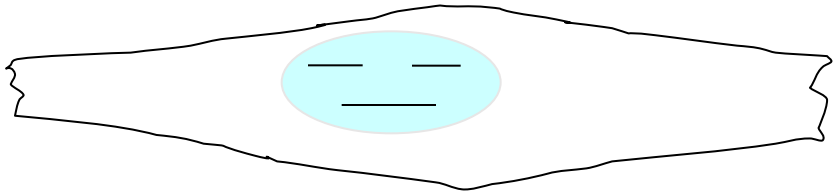
- Glutamate neurotransmitter binds to NMDA receptors
- Ca++ channels open causing Ca influx into cell
- Activation of calmodulin, which activates NOS
- Mechanism for start of synthesis dependent on body system
- NO synthesis takes place in endothelial cells, lung cells, and neuronal cells

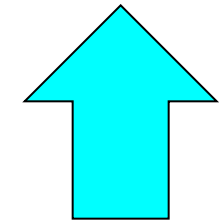
Nitric Oxide Signaling



1) Stimulated nerve releases ***Acetylcholine(ACh)*** at Nerve terminal

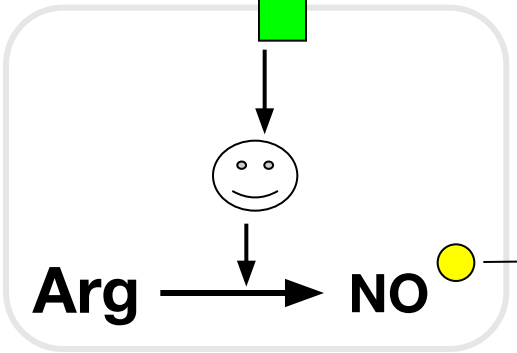
Relaxation of smooth muscle



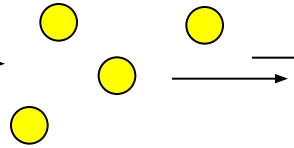


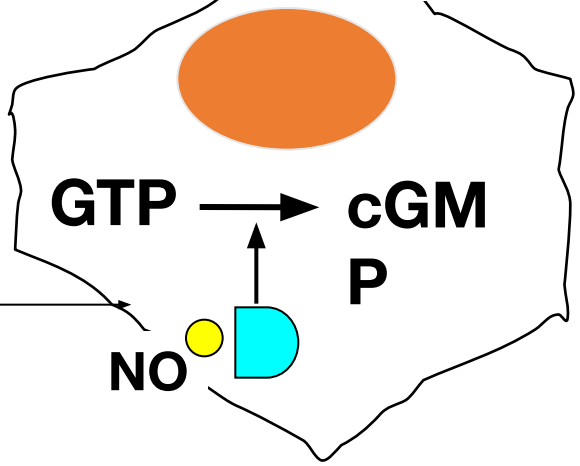
2) **ACh** binds to receptors on **endothelial cells**

Smooth muscle cell
blood vessel wall



4) NO *diffuses* across membranes



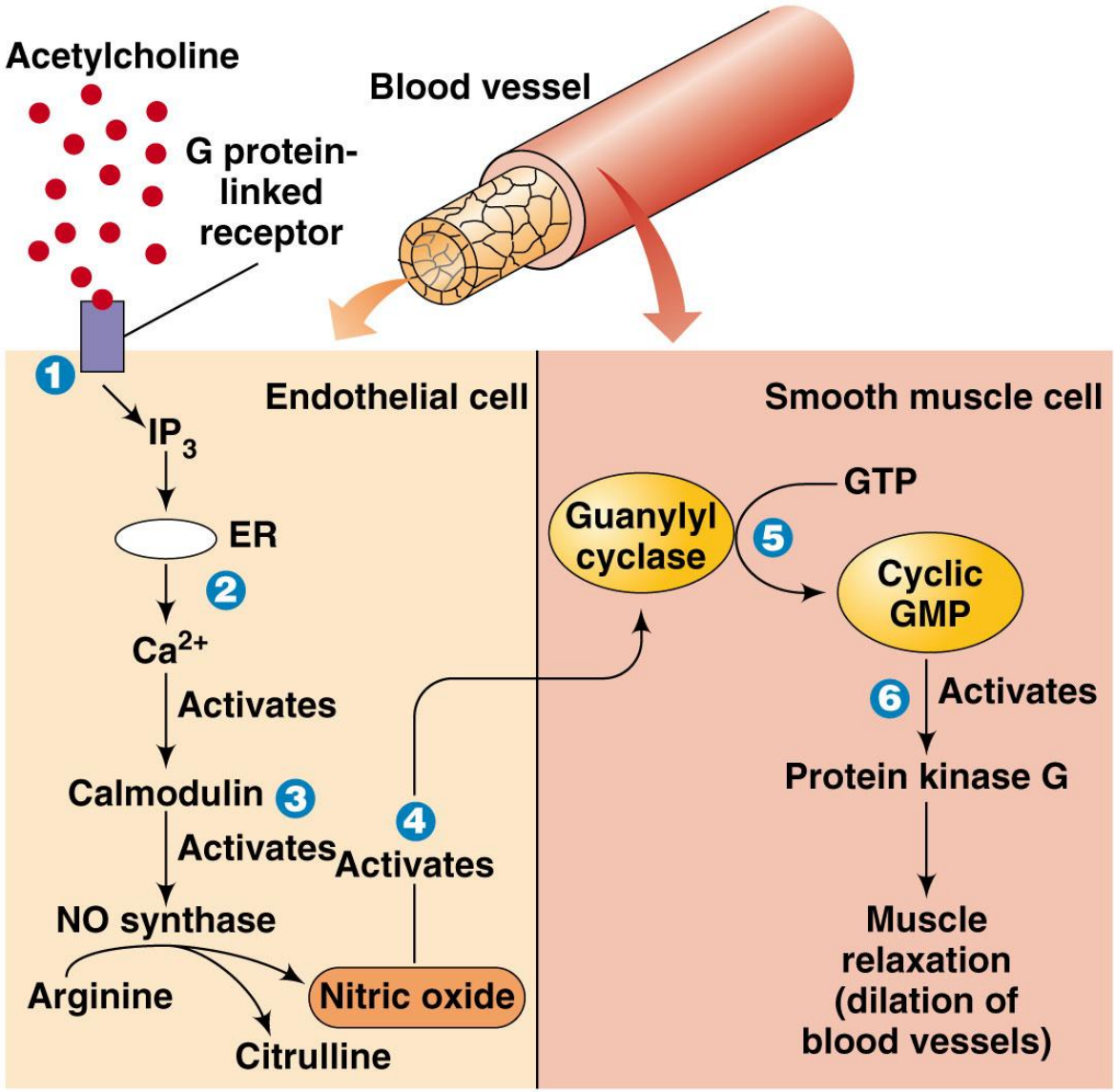


3) Activate ***NO synthase***

5) NO binds to ***Guanylyl cyclase***

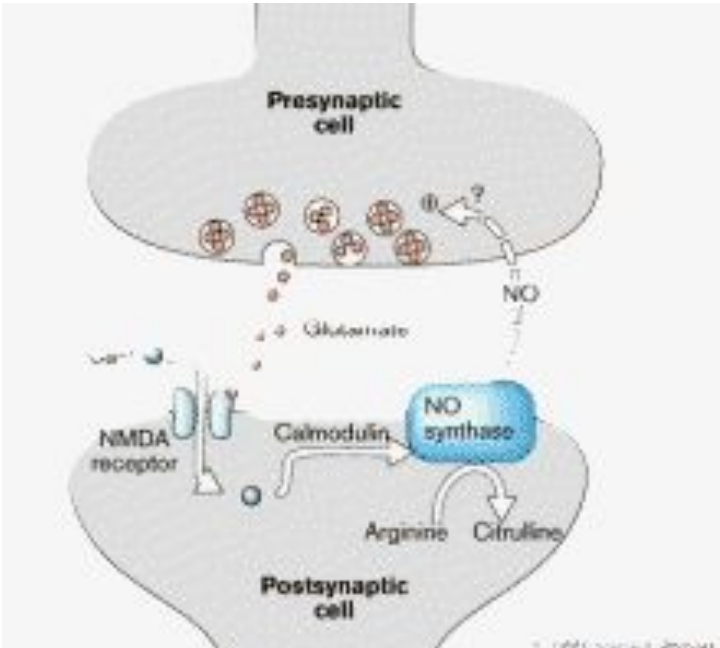
Nitric oxide as a Signal

Nitric oxide couples G protein-linked receptor stimulation in endothelial cells to relaxation of smooth muscle cells in blood vessels.
NO synthase converts arginine to citrulline and NO.
The binding of acetylcholine causes the release of NO in vascular endothelial cells that causes the relaxation of the vascular smooth muscle (vasodialator).
1) binding of acetylcholine to G protein receptors causes InsP3 production.
2) InsP3 releases calcium ions from endoplasmic reticulum.
3) ca++ ions and calmodulin form complex which stimulates NO synthase to produce NO.
4) NO (g) diffuses from endothelial cell into adjacent smooth muscle cells.
5) In smooth muscle cell, NO activates guanylyl cyclase to make cyclic GMP (cGMP).
6) cGMP activates protein kinase G which phosphorylates several muscle proteins to induce muscle relaxation.



The role of NO in Nervous System

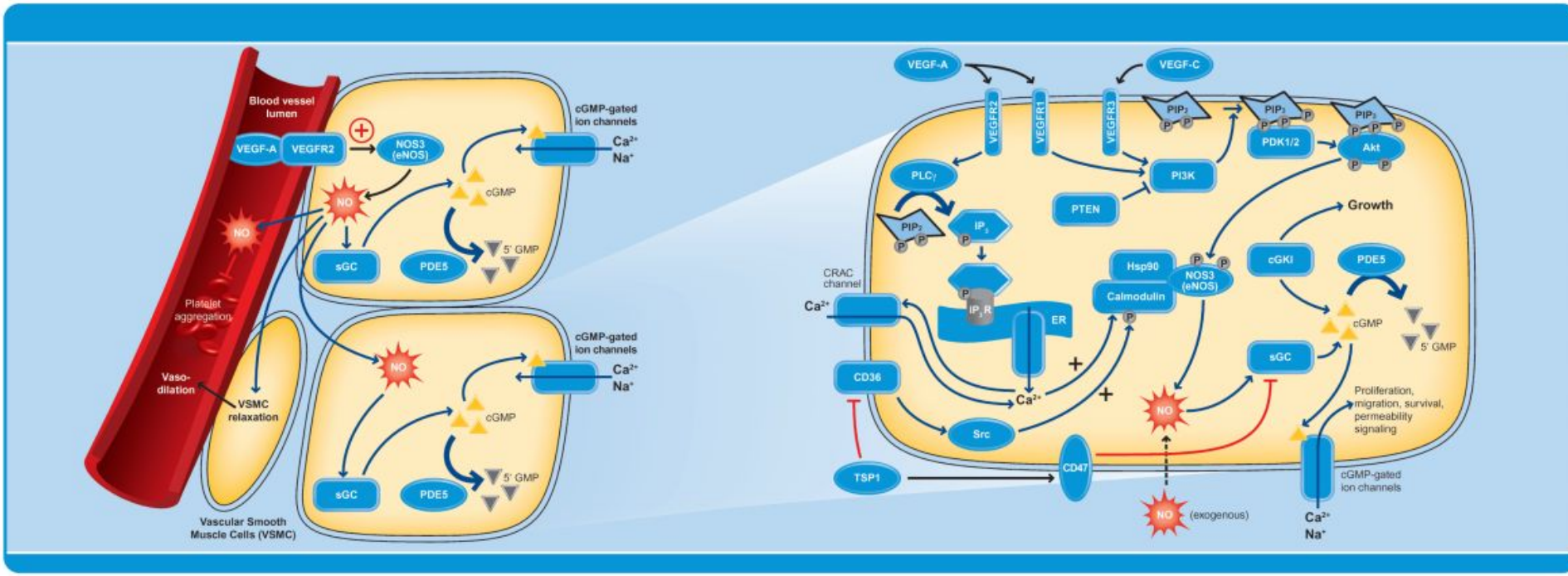
- It was found that nitric oxide activates the ejection process of neurotransmitters from nerve endings during synaptic transmission.
- Moreover, a molecule of nitric oxide can itself play the role of a neurotransmitter, that is, directly transmit a signal from one nerve cell to another.
- Not surprisingly, nitric oxide is present in all parts of the human brain: the hypothalamus, the midbrain, the cortex, the hippocampus, the medulla oblongata, and others.



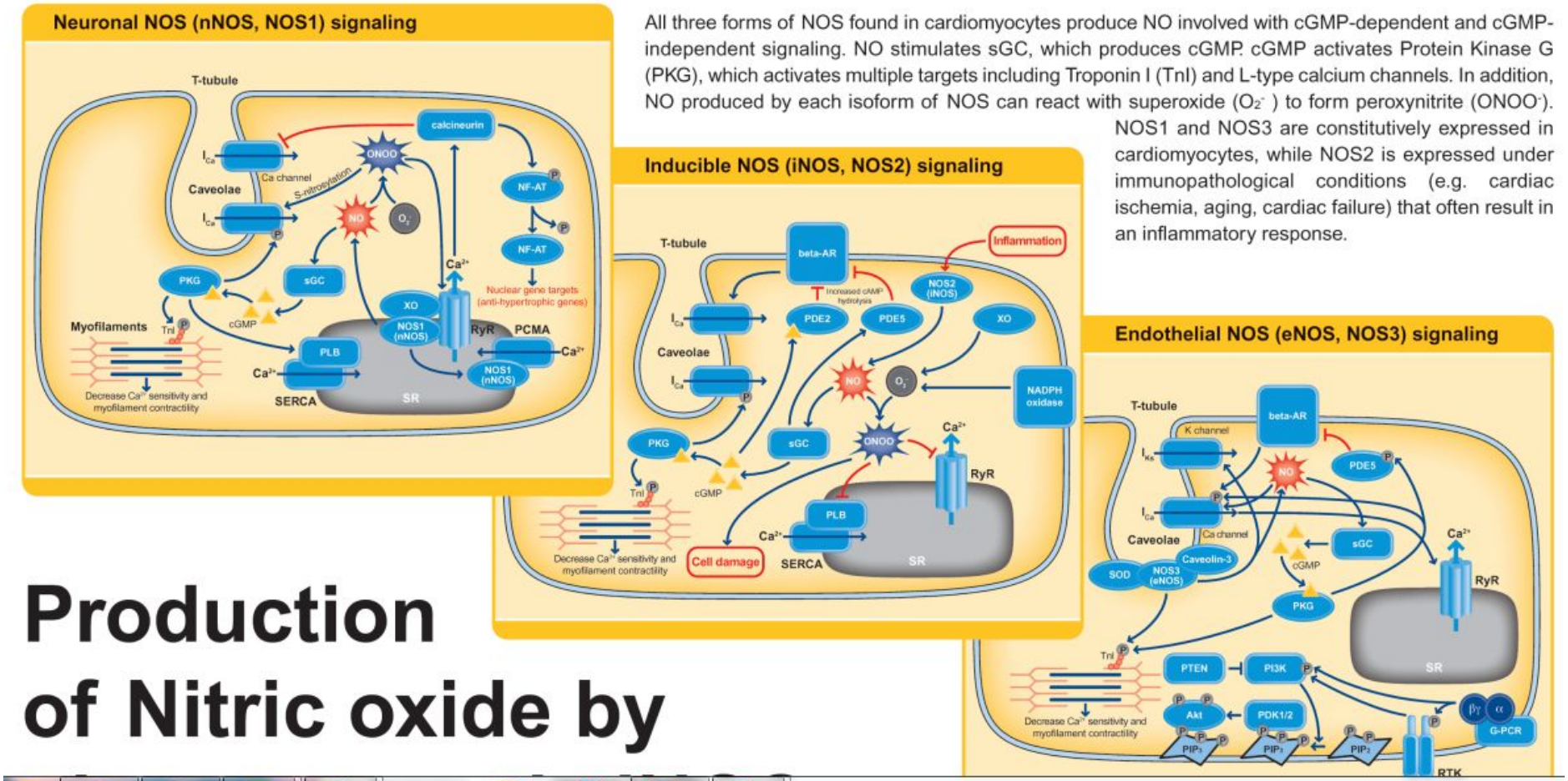
The role of NO in Cardio-Vascular System

- NO Regulates the relaxation of smooth muscle vessels and the synthesis of so-called "heat shock proteins" that "protect" the vessels in coronary heart disease.
- Inhibits the aggregation (clumping) of platelets, affects the transfer of oxygen by erythrocytes, as well as reactions involving chemically active molecules (free radicals) in the blood.

Nitric oxide signaling in endothelial cells



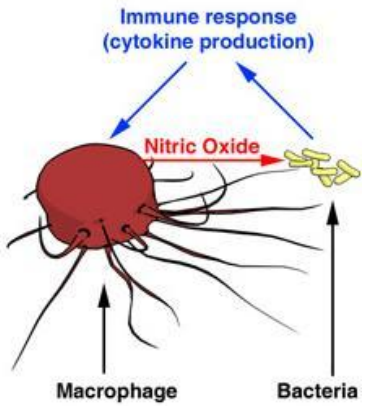
Nitric oxide signaling in cardiomyocytes



All three forms of NOS found in cardiomyocytes produce NO involved with cGMP-dependent and cGMP-independent signaling. NO stimulates sGC, which produces cGMP. cGMP activates Protein Kinase G (PKG), which activates multiple targets including Troponin I (TnI) and L-type calcium channels. In addition, NO produced by each isoform of NOS can react with superoxide (O_2^-) to form peroxynitrite ($ONOO^-$). NOS1 and NOS3 are constitutively expressed in cardiomyocytes, while NOS2 is expressed under immunopathological conditions (e.g. cardiac ischemia, aging, cardiac failure) that often result in an inflammatory response.

Production of Nitric oxide by

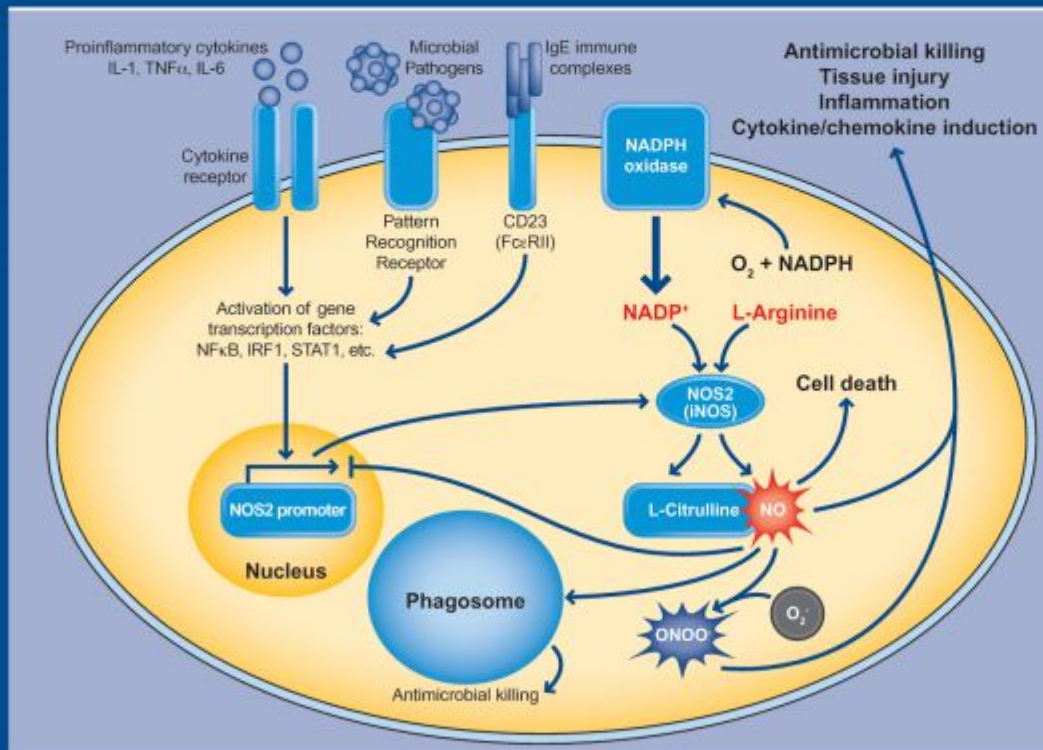
The role of NO in Immune System



- The activation of cells involved in the immune response - macrophages and neutrophils - is accompanied by the release of these cells by nitric oxide.

NO production by phagocytes such as macrophages occurs during oxidative burst, also known as respiratory burst. The major source of NO produced during oxidative burst comes from inducible nitric oxide (iNOS, NOS2).

NO participates in a number of reactions, including diffusing into the phagosome to mediate antimicrobial killing, or diffusing out of the cell to neighboring cells and tissues.



Pecularity of NO

- A characteristic feature of NO is the ability to diffuse quickly through the membrane of the cell synthesizing it into the intercellular space (in less than 5 seconds) and easily (without the participation of the receptors) to penetrate into the target cells. Inside the cell, it activates certain enzymes and inhibits others, thus participating in the regulation of cellular functions.
- In fact, nitrogen monoxide is a local tissue hormone.
- NO plays a key role in suppressing the activity of bacterial and tumor cells by either blocking some of their iron-containing enzymes, or by damaging their cellular structures with nitric oxide or free radicals formed from nitric oxide.

Peculiarity of NO..

- At the same time, a superoxide accumulates in the inflammatory focus, which causes damage to the proteins and lipids of the cell membranes, which explains its cytotoxic effect on the target cell. Consequently, NO, accumulating excessively in a cell, can act in two ways: on the one hand, it causes DNA damage and, on the other hand, has a antiinflammatory effect.

Pecularity of NO..

- Nitric oxide can initiate the formation of blood vessels. In the case of myocardial infarction, nitric oxide plays a positive role, as it induces a new vascular growth, but in cancer, the same process causes the development of tumors, promoting the nutrition and growth of cancer cells.
- On the other hand, as a result, the delivery of nitric oxide to tumor cells is improved. DNA damage due to NO is one of the reasons for the development of apoptosis (the programmed process of cellular "suicide", aimed at removing cells that have lost their functions).
- In the experiments, there was deamination of deoxynucleosides, deoxynucleotides and undamaged DNA upon exposure to a solution saturated with NO.
- This process is responsible for increasing the sensitivity of cells to alkylating agents and ionizing radiation, which is used in anticancer therapy.

Processes and Diseases with NO Participation

- Neurotransmission, memory, stroke
- Glaucoma and neural degeneration
- Vasodilation, blood pressure, blood flow
- Pulmonary hypertension
- Penile erection
- Angiogenesis, wound healing
- Atherogenesis
- Inflammation, arthritis, nephritis, colitis, etc
- Cytotoxicity tissues, pathogens, tumors

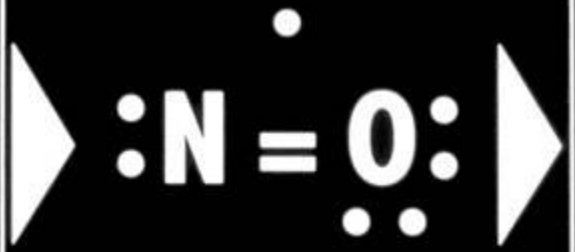
Processes and Diseases with NO Participation

- Tissue transplantation
- Septic shock, dyalitic hypotension
- Platelet aggregation
- Gastrointestinal motility
- Hormone secretion
- Gene regulation
- Hemoglobin delivery of oxygen
- Stem cell proliferation and differentiation
- Bronchodilation

Some Sources of Nitric Oxide and Some of its Effects

Some Sources of Nitric Oxide

- Natural substances in the body
- Drugs
- Car Exhaust
- Cigarette Smoke
- Fires
- Other combustible materials



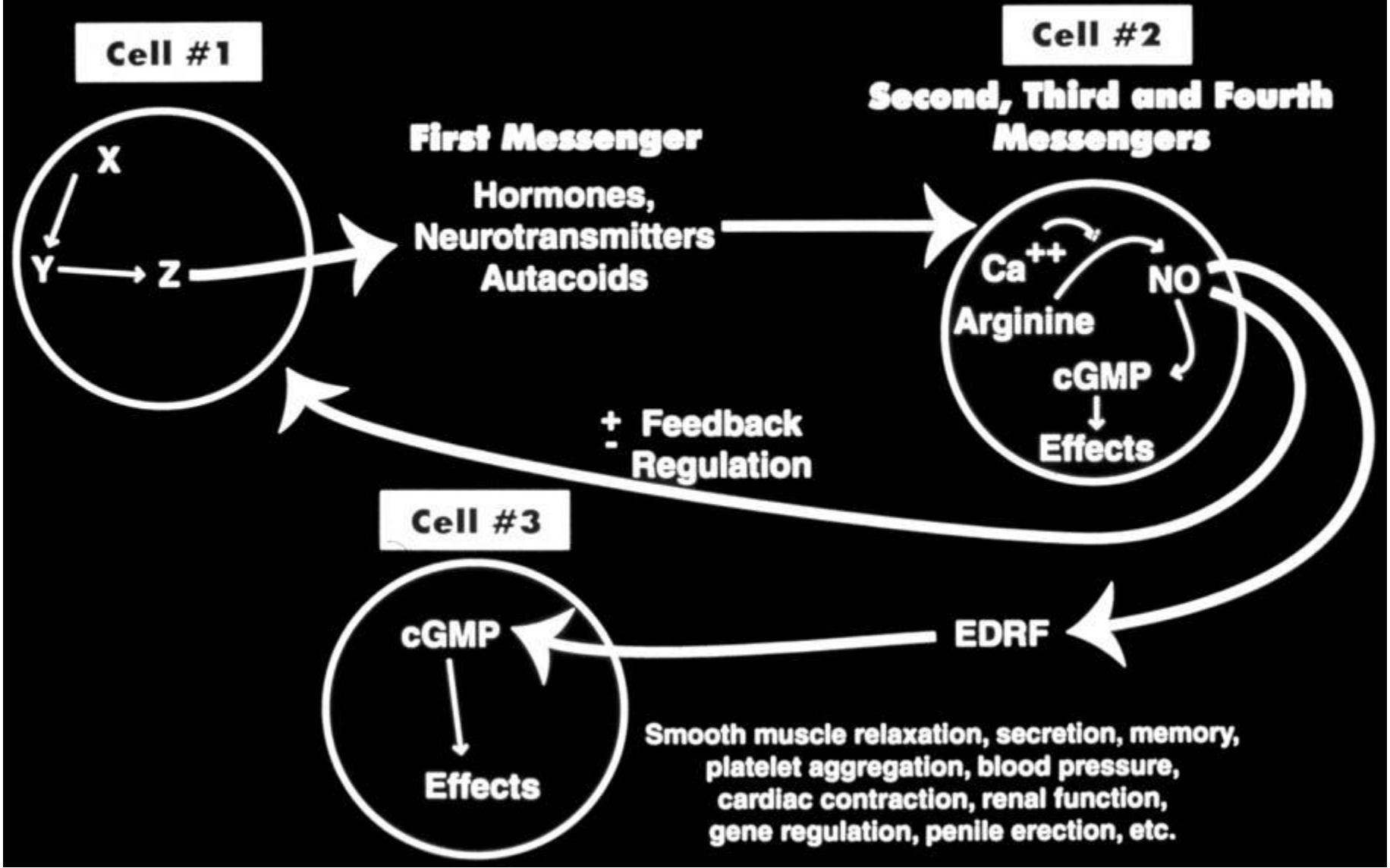
Nitric Oxide

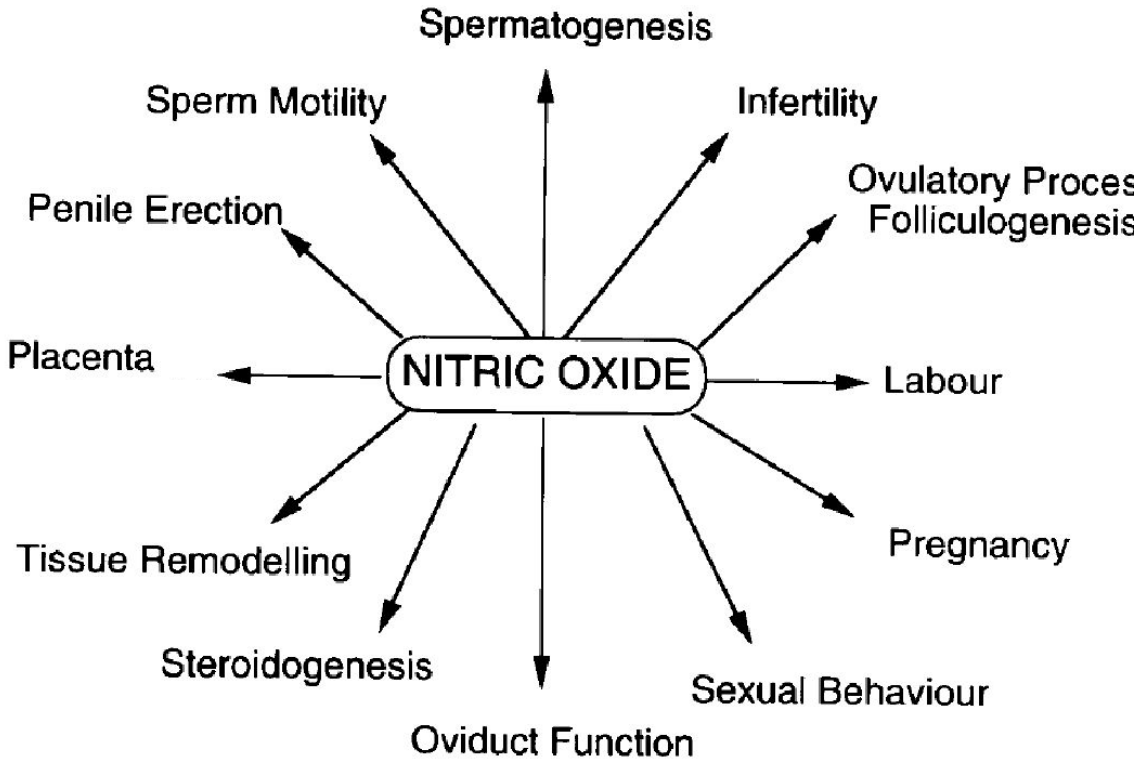
A reactive free radical with an unshared electron

Some Effects

- Ozone depletion and earth warming
- Free radical interactions
- Numerous biological effects (both beneficial & deleterious
  - Blood pressure
  - Memory
  - Stroke
  - Diabetes
  - Esophagitis
  - Anti-bacterial
  - Platelet aggregation
  - Arthritis
  - Inflammation
  - Angina
  - Heart contraction
  - Penile erection
  - Gene regulation
  - Etc.

The Nitric Oxide and Cyclic GMP Cell Signaling Pathway (Cells talking to each other)




Spermatogenesis
Sperm Motility
Infertility
Ovulatory Proces
Folliculogenesis
Penile Erection
Placenta
NITRIC OXIDE
Labour
Tissue Remodelling
Pregnancy
Steroidogenesis
Sexual Behaviour
Oviduct Function

Conclusion

- NO is a universal messenger molecule
- It is involved in a wide variety of pathophysiogical and biochemical reactions.
- In summary NO is involved in regulation of B.P., prevention of aggregation and adhesion of platelets, promotion of penile erection.
- Other way to increase active concentration of endogenous NO such as by prolonging its half life of duration of its actions.
- NO donating compounds can be used as replacement therapy to treat its impaired production
- NO also as therapeutic potential for Ischemic CVS diseases, pulmonary hypertension associated with cardiac and respiratory diseases.
- They are far from ideal because of the associated side effect mainly due to the catabolism of NO into NO2
- Therefore a technology to regulate in vivo synthesis of NO by genetic manipulation would be a welcome move.