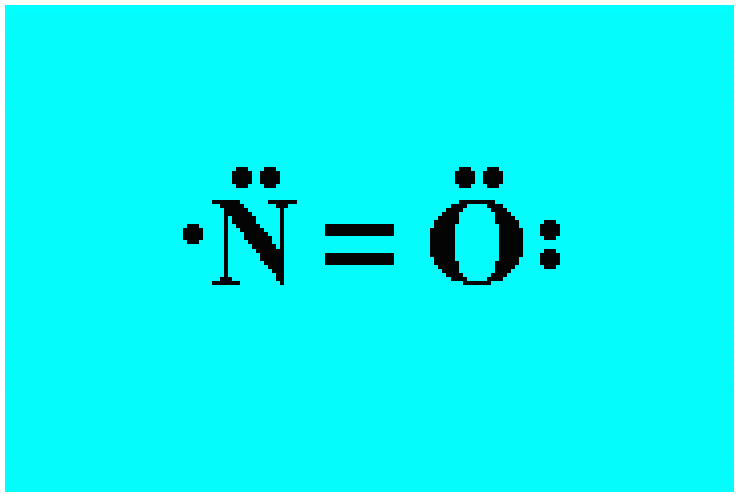
Syntasy oxidu dusnatého

Antonín Lojek

Oxid dusnatý (= nitric oxide = NO)



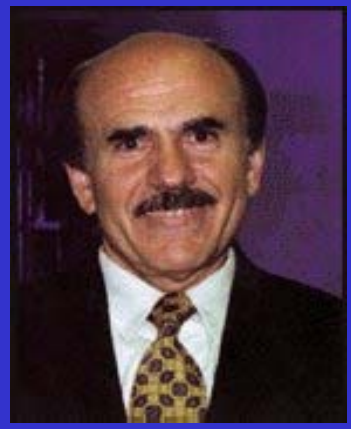
NO je molekulou složenou z 1 atomu kyslíku a 1 atomu dusíku

Tyto atomy jsou vázány dvojnou vazbou

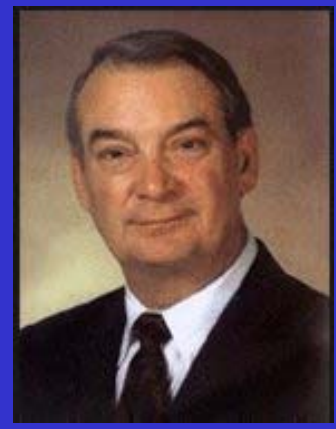
Na atomu kyslíku se nacházejí 2 páry elektronů (nevazebných)

Na atomu dusíku se nachází 1 pár nevazebných elektronů a jeden elektron nepárový

Robert F Furchgott
1916
Dept. of Pharmacology,
SUNY Health Science Center
New York



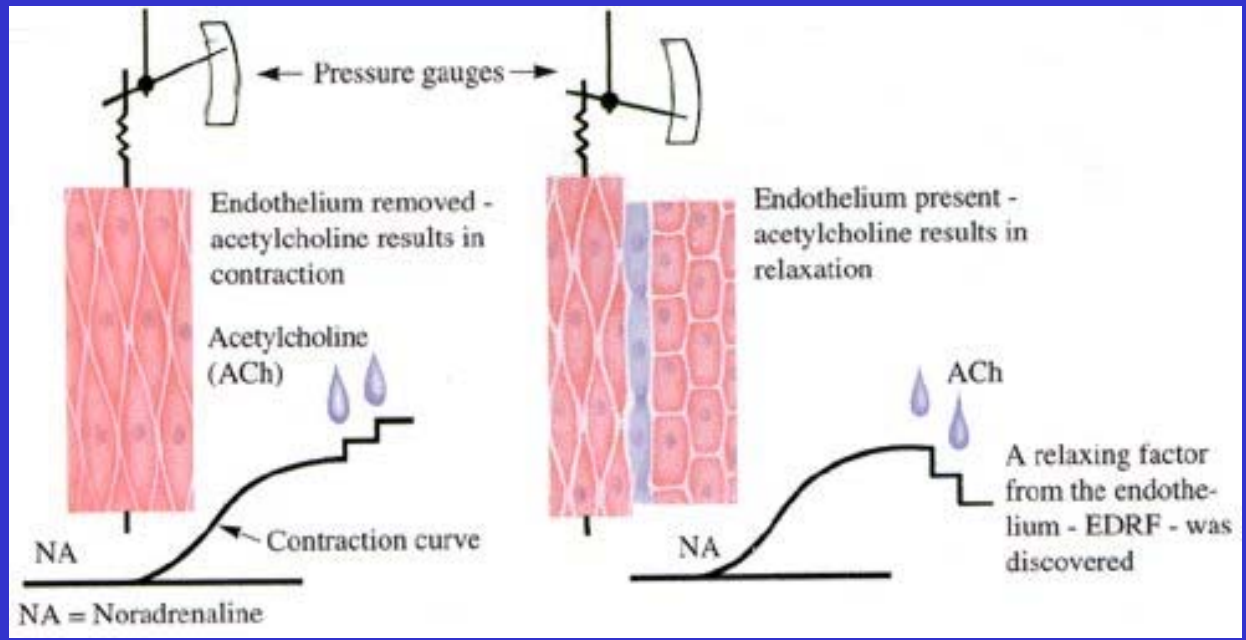
Louis J Ignarro,
1941
Dept. of Molecular and Medical Pharmacology
UCLA School of Medicine
Los Angeles



Ferid Murad
1936
Dept. of Integrative Biology Pharmacology and Physiology
University of Texas Medical School, Houston

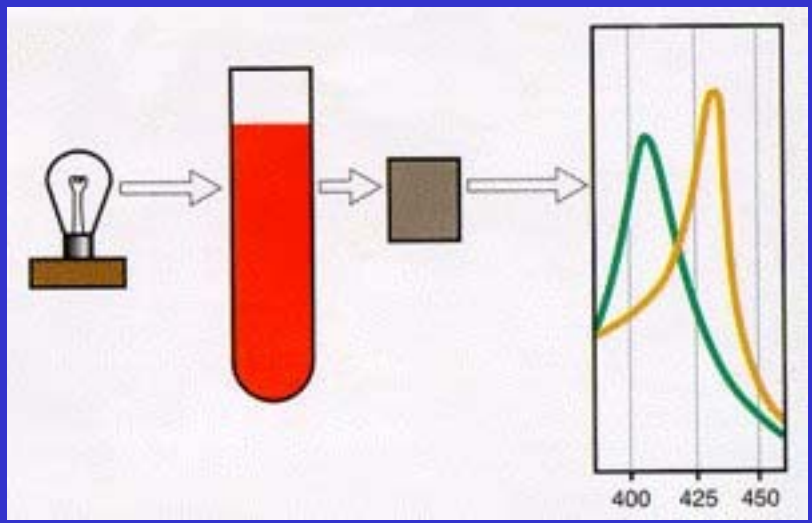
Furchgottův sandwich

Furchgott prokázal, že relaxace cév indukovaná acetylcholinem je závislá na endoteliu.
Použil dva kousky aorty, u jednoho odstranil epitelium

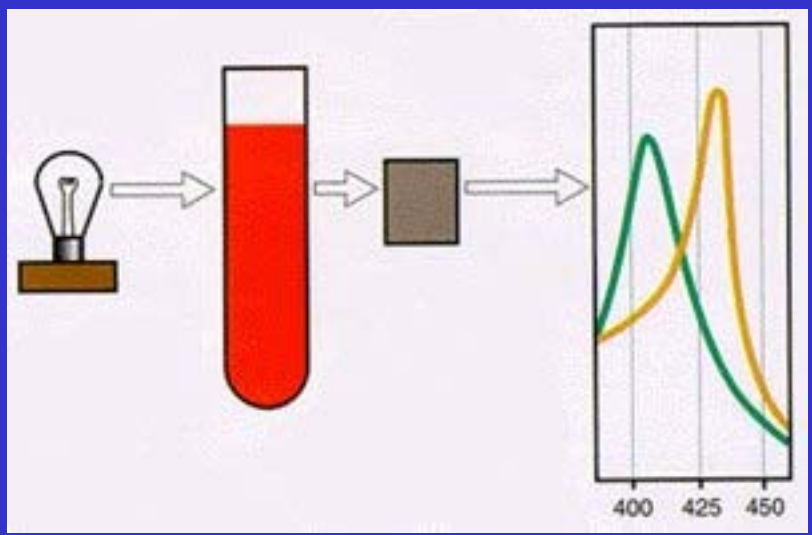


Ignarrova spektrální analýza

Ignarro pomocí spektrální analýzy prokázal, že EDRF je totožný s NO.



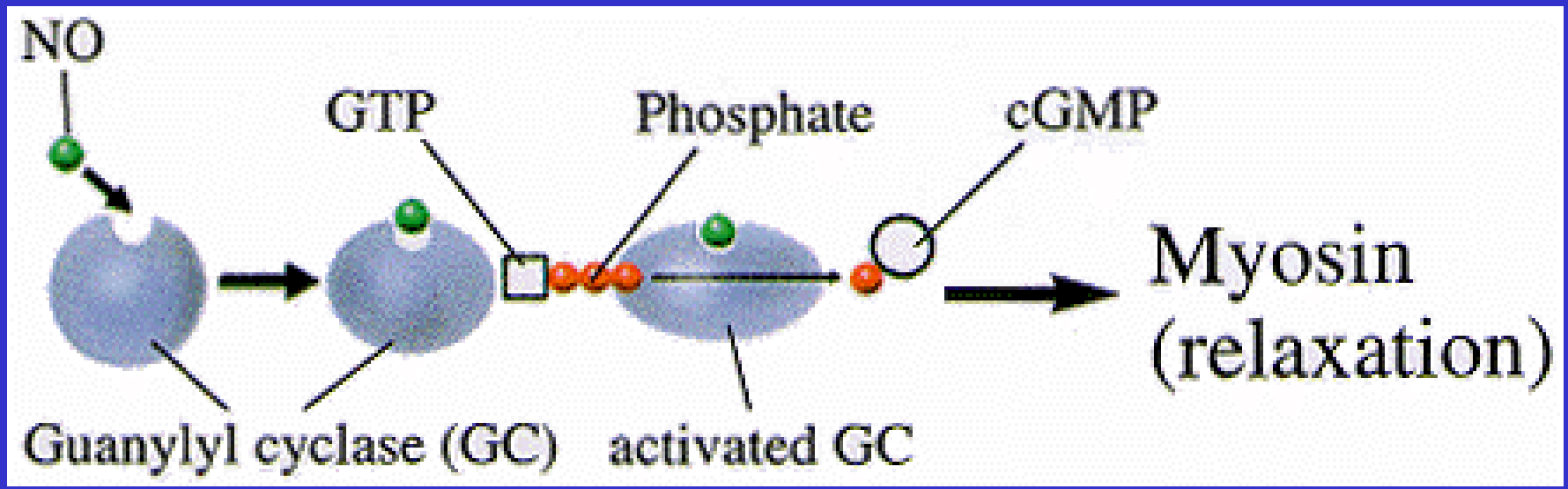
Hemoglobin (žlutý) exponovaný endoteliálním buňkám produkujícím EDRF
(konverze oxyhemoglobinu na methemoglobin)



Hemoglobin (žlutý) exponovaný přímo NO
Posun v absorbční křívce je identický (EDRF = NO)

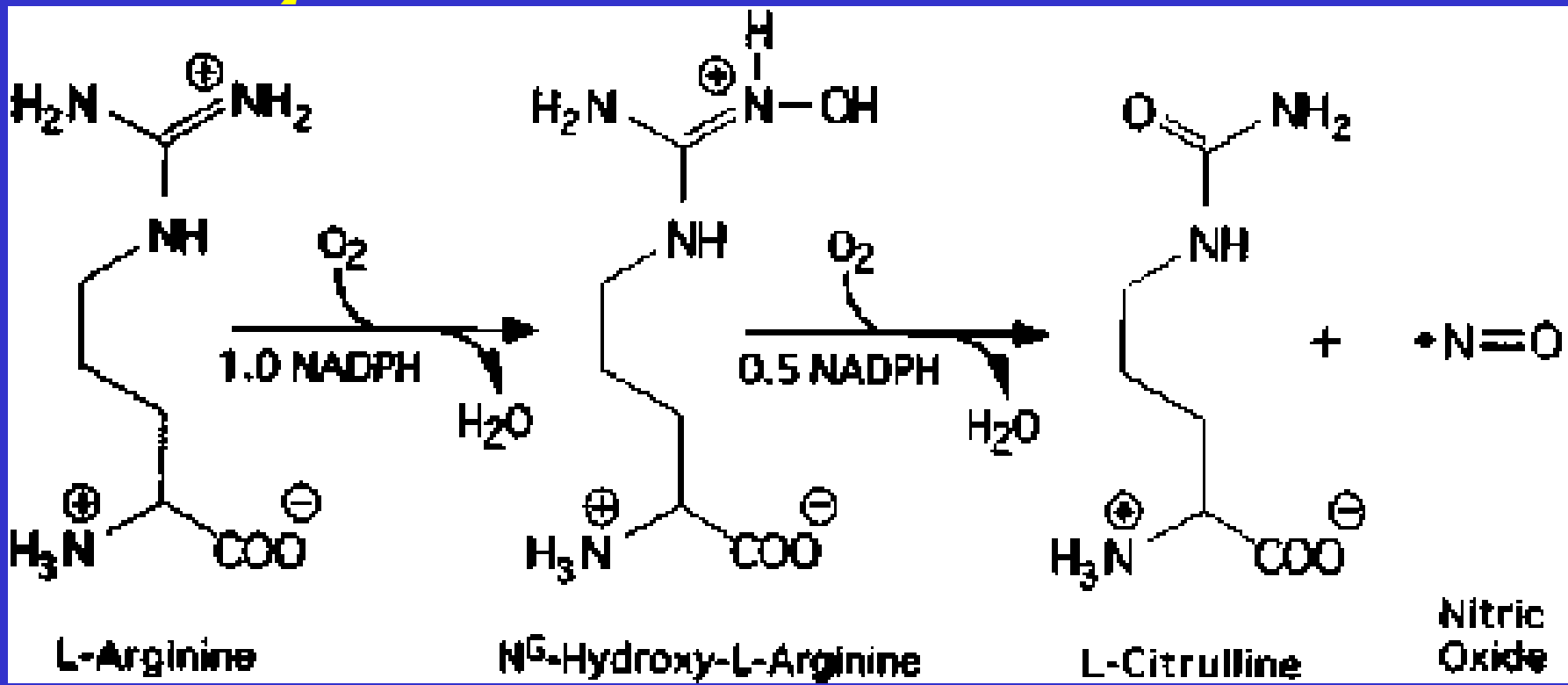
Muradova enzymatická aktivace

Murad věděl, že nitroglycerin působí relaxaci hladké svaloviny.
Enzym guanylát cyklasa byla aktivována a indukovala zvýšení cGMP s následnou ralaxací svalu.
Působí nitroglycerin cestou uvolňování NO ???
Probublával NO přes tkáň obsahující enzym – cGMP se zvyšoval.



V savčích buňkách je NO tvořen oxidací terminálního guanidino dusíku L-argininu molekulárním kyslíkem; kromě NO vzniká L-citrulin

L-arg + O_2 --> NO + L-cit



Celou komplexní reakci katalyzuje jediný enzym, NO syntáza, která existuje ve 3 isoformách

Syntásy oxidu dusnatého

- **neuronální** syntása oxidu dusnatého (NOS1 = nNOS)
- **inducibilní** syntása oxidu dusnatého (NOS2 = iNOS)
- **endotheliální** syntása oxidu dusnatého (NOS3 = eNOS)

Každá z těchto syntás:

- má rozdílnou tkáňovou distribuci
- lokalizovaná na různých chromozomech

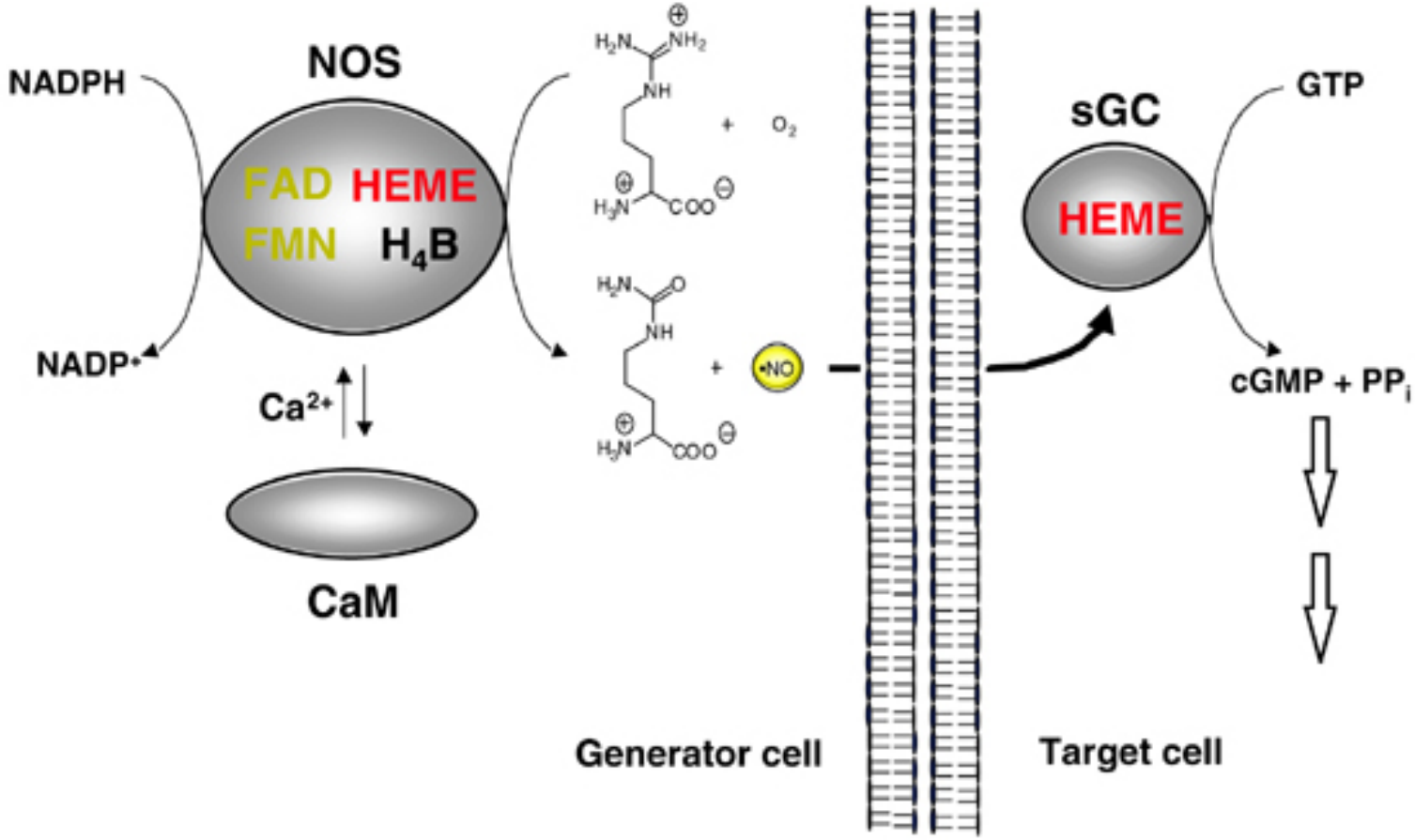
Všechny 3 isoformy NO syntázy:

jsou aktivní jako homodimery

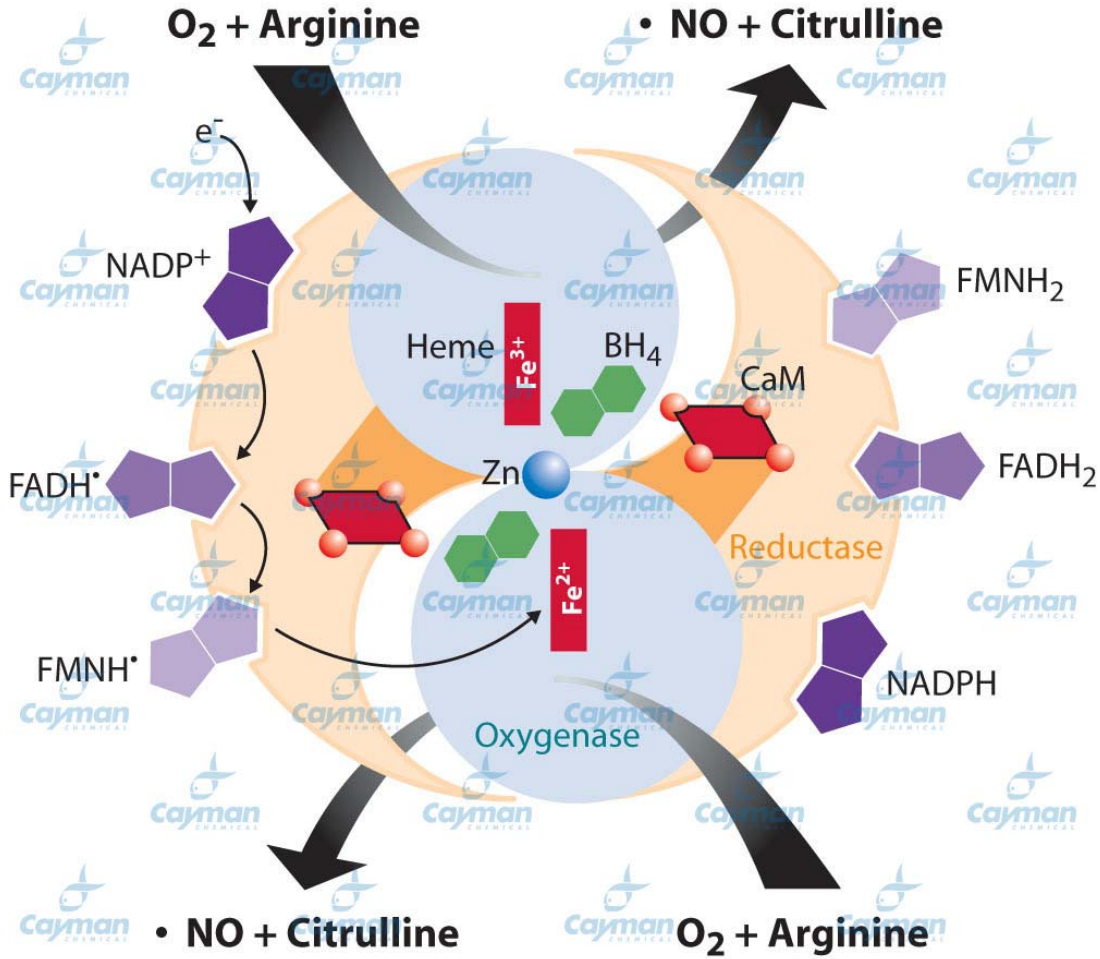
obsahují v aktivním centru hem

jsou **stereospecifické** (D-arginin není substrátem)

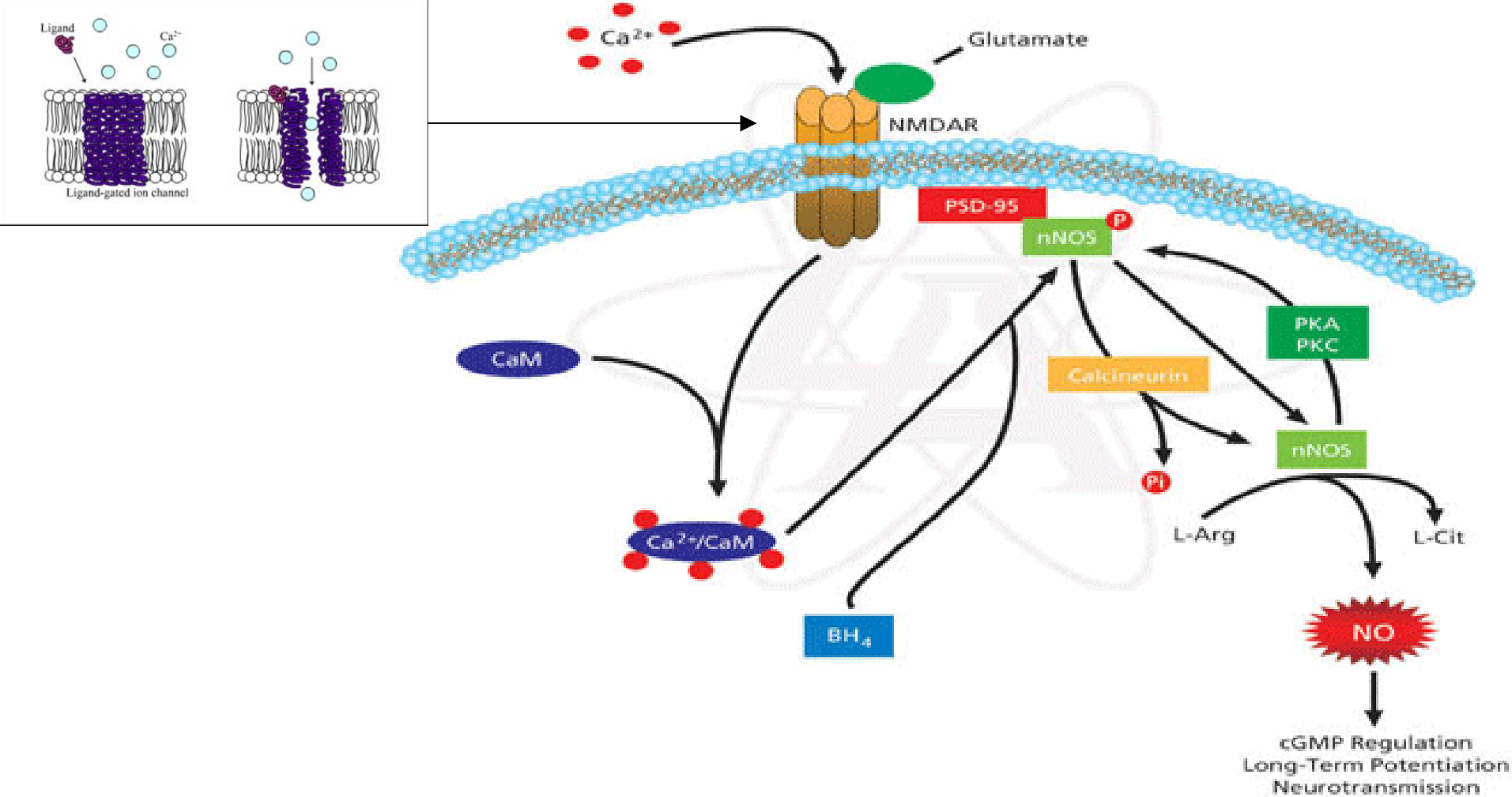
jako **kofaktory** vyžadují:
NADPH, 6(R)-5,6,7,8-tetrahydrobiopterin, FAD, FMN a kalmodulin (ten se k NOS typu I a III váže po navázání Ca na kalmodulin, NOS II váže kalmodulin trvale)



Nitric oxide synthase (NOS) catalyzes the conversion of L-arginine to NO and citrulline. The critical biological role of NO is now well-established, both in signal transduction and in the host response to infection (1, 2). In signal transduction it serves as a cell-to-cell signaling agent involving stimulation of the synthesis of the second messenger guanosine 3':5'-cyclic monophosphate (cGMP) in the target cell, as illustrated.

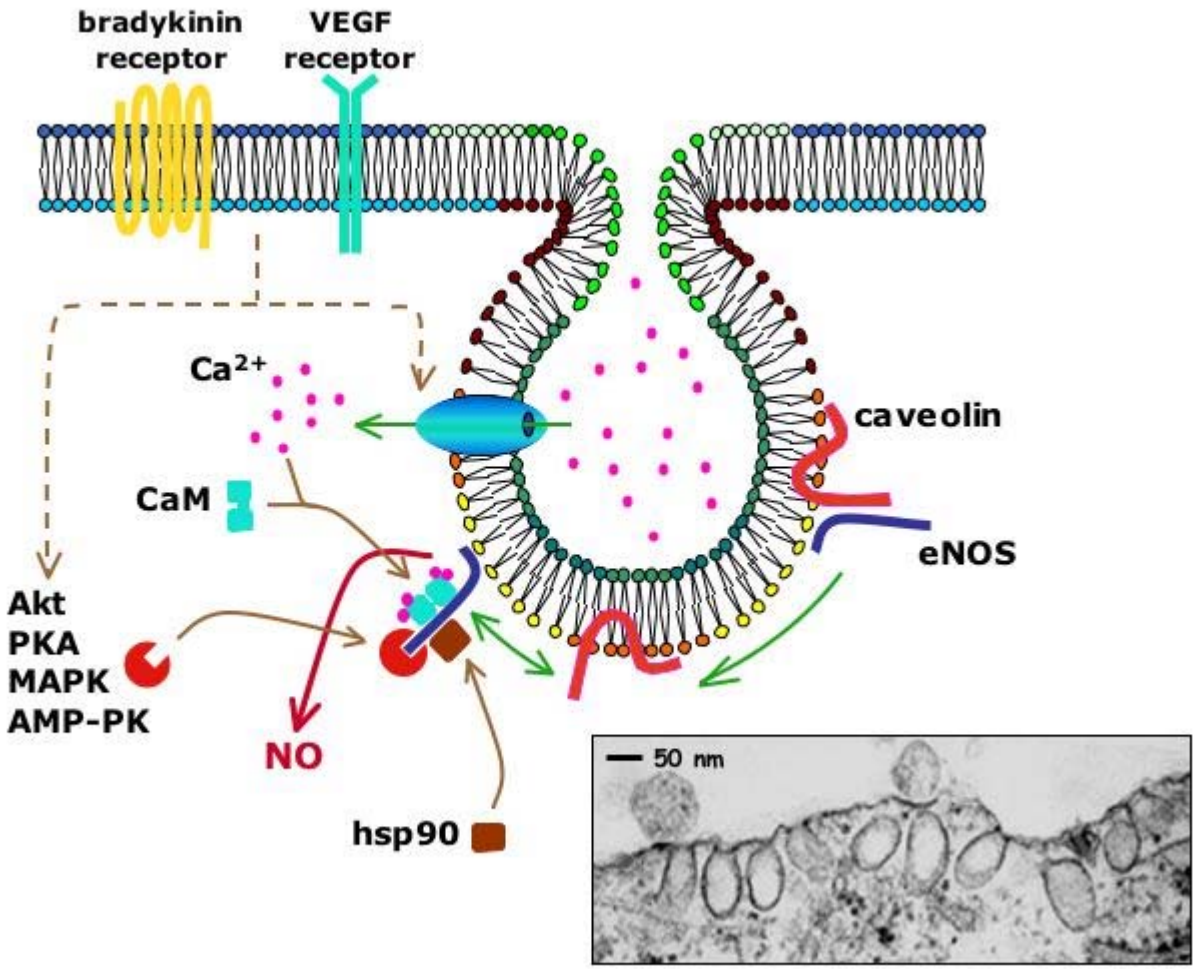


The NOS homodimer is shown with the N-terminal oxygenase domain of each monomer in gray and the C-terminal reductase domain in beige. Cofactors in their oxidized state are shown in the left hand monomer, and reduced cofactors are shown on the right. Substrates O2 and arginine bind at or in close proximity to the heme iron. The conversion to products NO and citrulline is a multi-step process involving at least one distinct intermediate, N-hydroxy arginine. Electron flow proceeds from NADPH through the flavin nucleotides (purple) of the reductase domain to the heme (red) on the other monomer. It is unclear whether BH4 (green) participates as an active component of the electron transport chain. A zinc atom is tetrahedrally coordinated to 2 cysteines from each subunit in the active dimer. Four calcium ions (red) are shown coordinated to Calmodulin (CaM) at a bridge point between the oxygenase and reductase domains. The dimer interface occurs at large portions of the oxygenase domain of the monomers and involves BH4, Ca2+/CaM, and Zn as active stabilizing molecules.



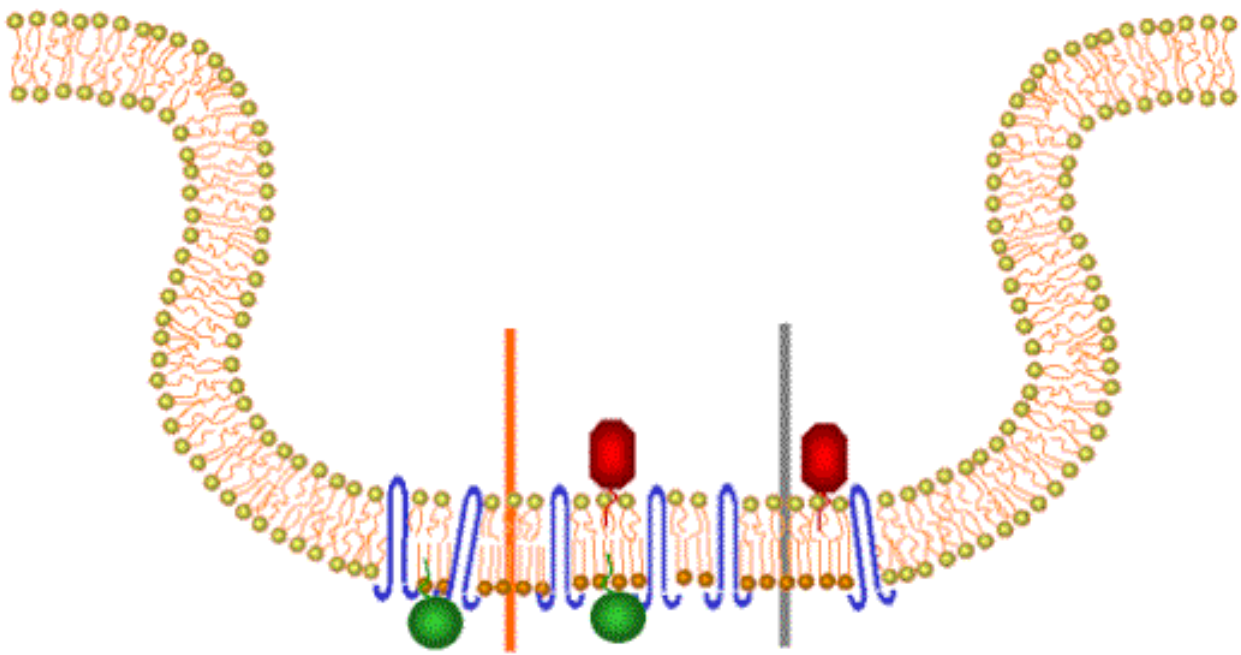
Neuronal Nitric Oxide Synthase (nNOS)

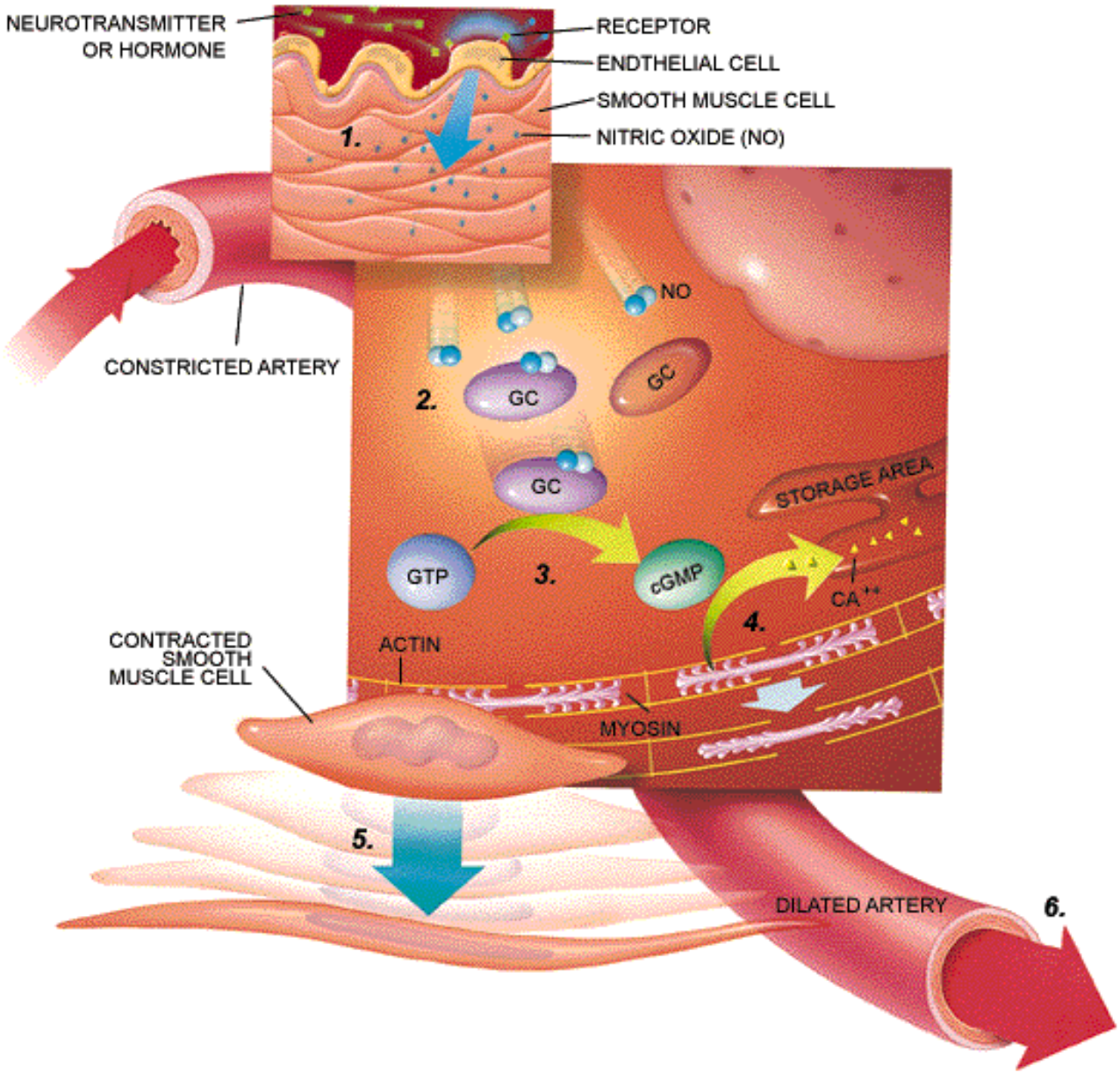
Three isoforms of nitric oxide synthase (NOS) have been identified. All are homodimers with subunits of 130-160 kDa. All have binding sites for NADPH, FAD, and FMN near the carboxyl terminus (the reductase domain), and binding sites for tetrahydrobiopterin (BH_4) and heme near the amino terminus (the oxygenase domain). The reductase and oxygenase domains are linked by a calmodulin (CaM) binding site. Occupation of this site facilitates electron transfer from the cofactors in the reductase domain to heme during nitric oxide production. NOS catalyzes the conversion of arginine to citrulline and nitric oxide (NO). Neuronal nitric oxide synthase (nNOS, bNOS, cNOS, Type I) is associated with the post-synaptic density protein (PSD-95) in the neuronal membrane. In response to increased intracellular Ca^{2+}, nNOS interacts with CaM. The Ca^{2+}-CaM complex, in combination with BH_4, binds to nNOS and induces its translocation from the plasma membrane to the cytoplasm. The dephosphorylation of nNOS by calcineurin initiates the production NO. NO activates guanylyl cyclase (GC) and activates the various cGMP-regulated signaling pathways. nNOS is inactivated by phosphorylation by protein kinase A (PKA) or protein kinase C (PKC).



Endothelial nitric oxide synthase is localised to caveolae. Endothelial nitric oxide synthase (eNOS) is a lipid raft/caveolar protein apparently regulated by caveolin. Agonist stimulation induces calcium dependent association of protein cofactors and kinases ultimately resulting in generation of nitric oxide from Arginine.

Caveolae are specialized lipid rafts that perform a number of signalling functions (Reviewed, Anderson, 1998). Caveolae were first identified by EM examination in the mid 50' by two workers (Palade, 1953; Yamada, 1955), as 50-100nm "flask shaped" invaginations of the plasma-membrane. They are found in a variety of cell types especially endothelial cells, but none exist as classical invaginated caveolae in neuronal tissues. Many proteins and lipids are known to be enriched in caveolae (see table 1), and labelling of cells with a PH domain protein marker for PIP_2, indicates that this lipid is not concentrated in caveolae (Watt *et al*, 2002). **Caveolin** (Rothberg *et al*, 1992), is a principle marker of the caveolae.




NEUROTRANSMITTER OR HORMONE
RECEPTOR
ENDTHELIAL CELL
SMOOTH MUSCLE CELL
NITRIC OXIDE (NO)
1.
CONSTRICTED ARTERY
NO
GC
GC
2.
GC
STORAGE AREA
GTP
3.
cGMP
CA ++
4.
CONTRACTED SMOOTH MUSCLE CELL
ACTIN
MYOSIN
5.
DILATED ARTERY
6.

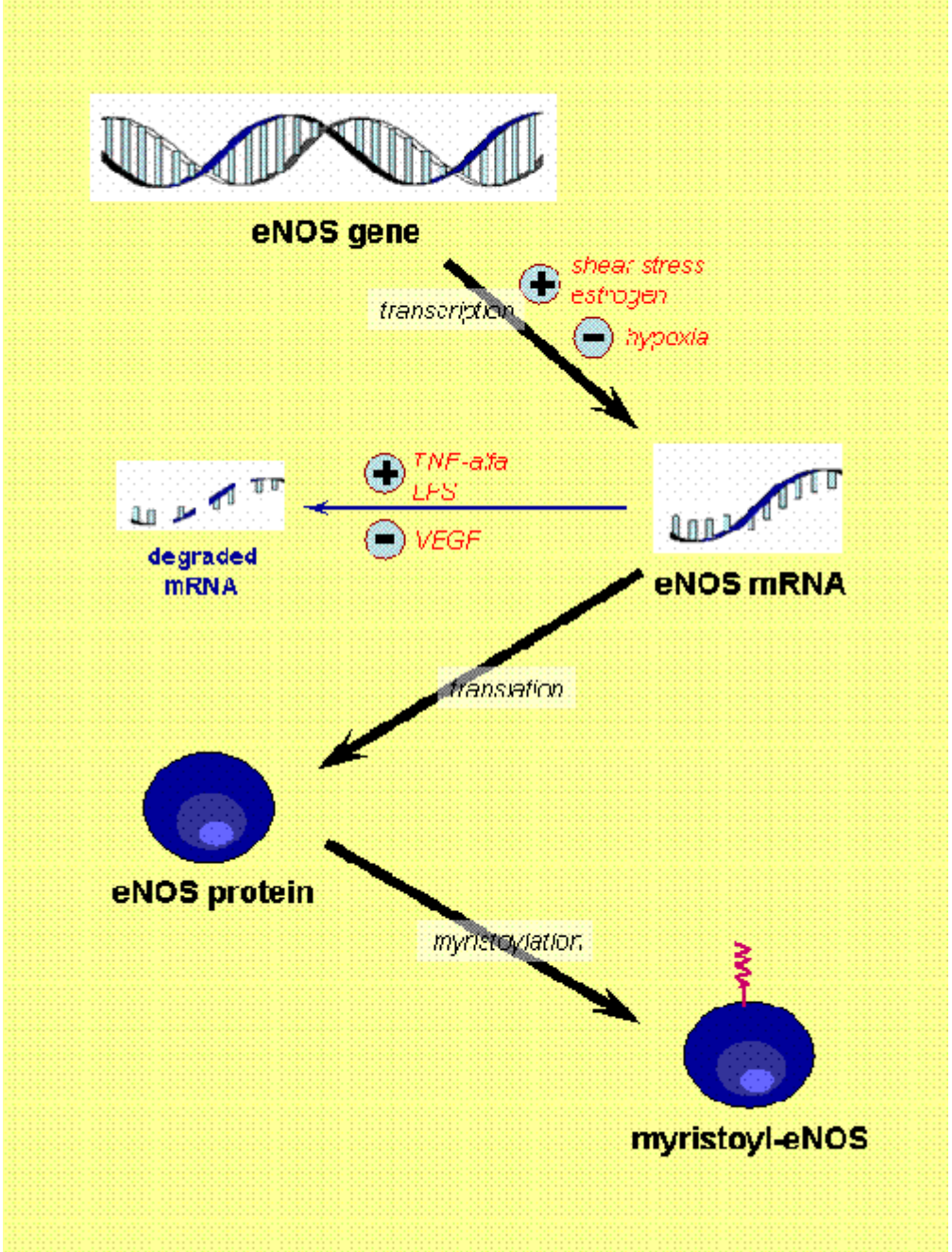


Fig. 1. eNOS regulation (part I). [Based on Govers and Rabelink, Am J Physiol 2001, 280:F193].
Here, the expression of eNOS and permanent changes of the protein (e.g. myristoylation) are shown. There are several factors that regulate the transcription of eNOS gene (shear stress, estrogen and hypoxia) and others that modulate the stability of its mRNA (tumor necrosis factor alfa or TNF-alfa, lipopolysacharide or LPS, and vascular endothelial growth factor or VEGF). Myristoylation seems a critical factor to allow the final location of the enzyme at certain specific domains of the membrane.

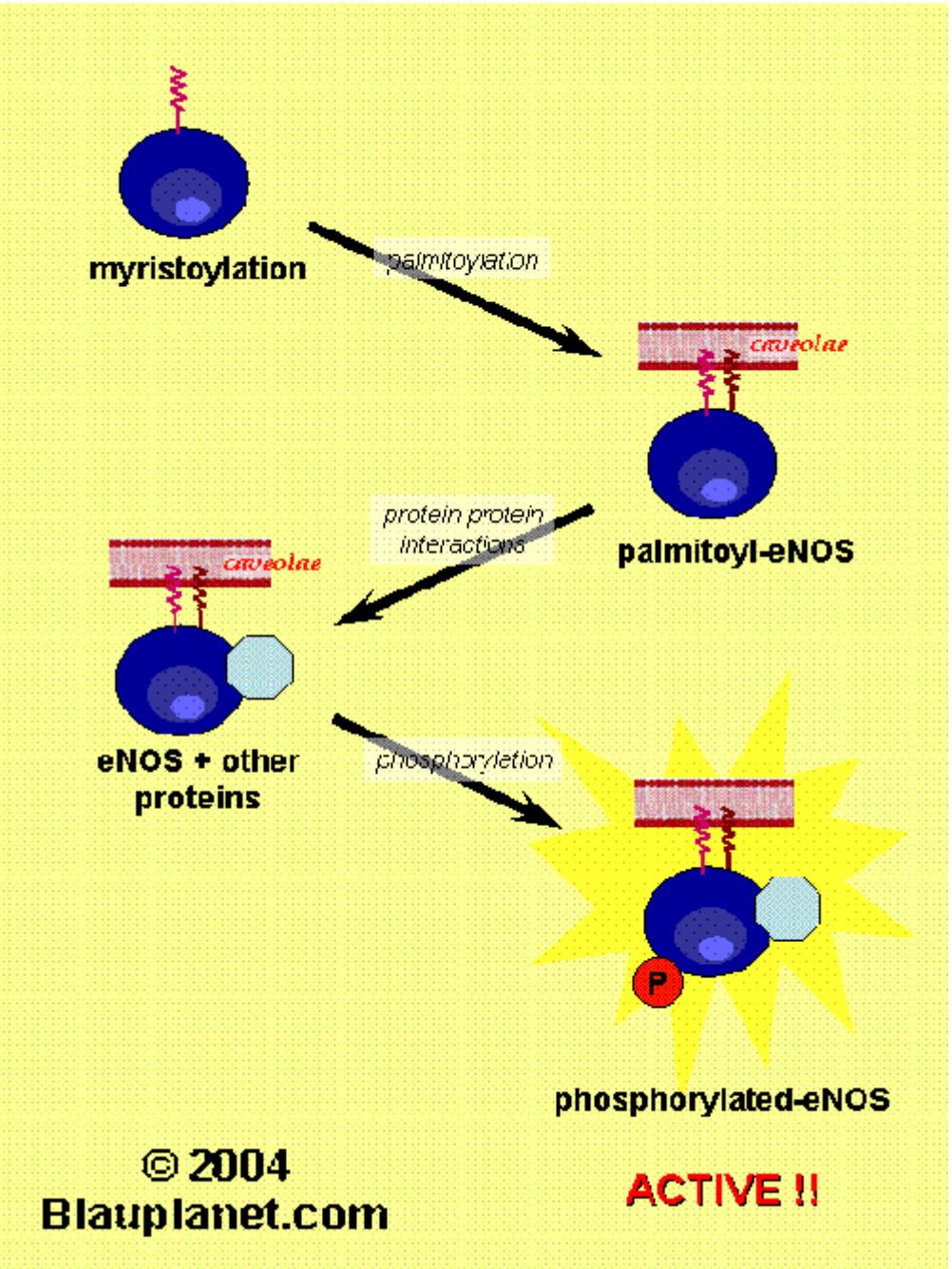
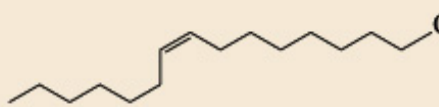
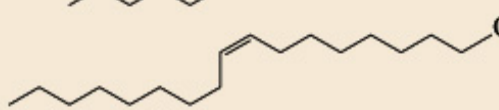
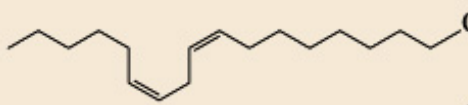
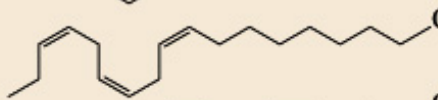
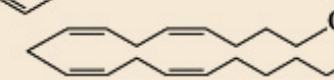
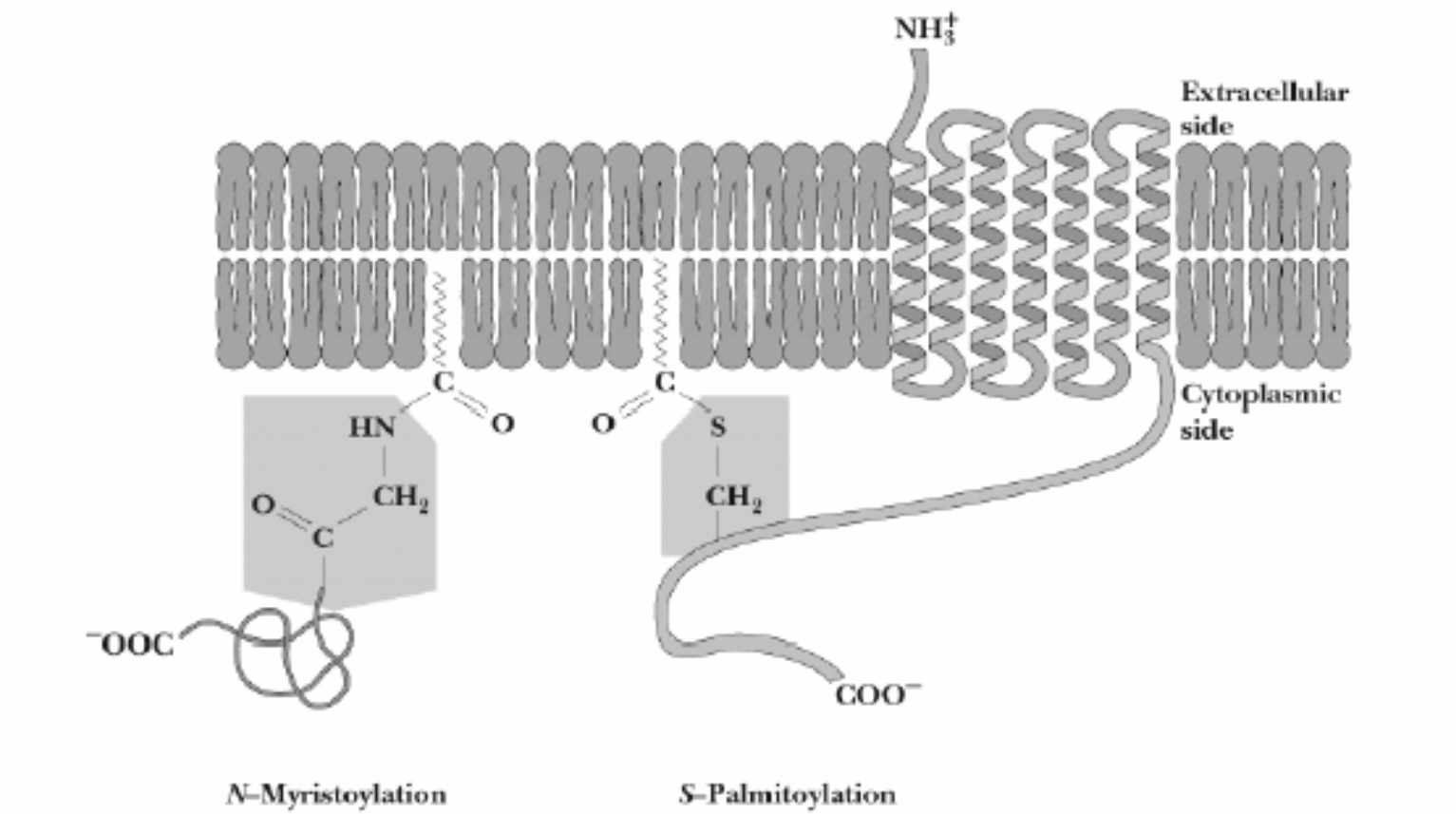


Fig. 2. eNOS regulation (part II). [Based on Govers and Rabelink, Am J Physiol 2001, 280:F193].
Besides myristoylation, eNOS protein suffers another changes such as palmitoylayion, phosphorylation and specific interactions with another proteins. After those modifications the eNOS protein is active and synthetizes NO or in some cases superoxide ion (this later circunstance can take place when the substrate, L-arginine, or tetrahydrobiopterin are deficient and has pathophysiological consequences). Then, all these non-permanent modifications of eNOS revert and eNOS is deactivated. A cycle of activation-deactivation occurs in parallel with a cycle of association and dissociation from the caveoale at the plasma membrane.

TABLE 24.1 Common Naturally Occurring Fatty Acids

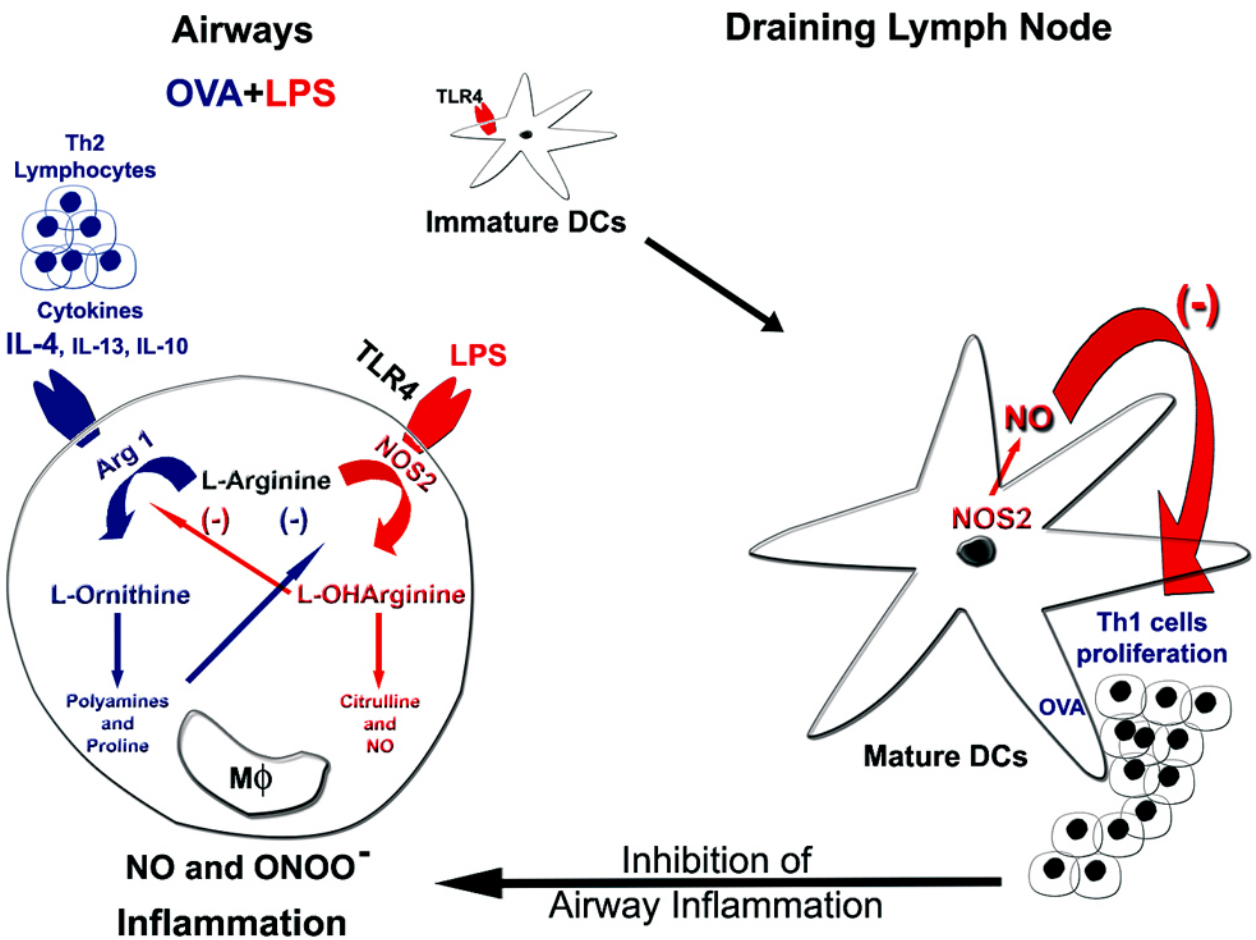
Number of carbons	Common name	Systematic name	Structure	Melting point °C
Saturated				
12	lauric acid	dodecanoic acid		44
14	myristic acid	tetradecanoic acid		58
16	palmitic acid	hexadecanoic acid		63
18	stearic acid	octadecanoic acid		69
20	arachidic acid	eicosanoic acid		77
Unsaturated				
16	palmitoleic acid	(9Z)-hexadecenoic acid		0
18	oleic acid	(9Z)-octadecenoic acid		13
18	linoleic acid	(9Z,12Z)-octadecadienoic acid		−5
18	linolenic acid	(9Z,12Z,15Z)-octadecatrienoic acid		−11
20	arachidonic acid	(5Z,8Z,11Z,14Z)-eicosatetraenoic acid		−50
20	EPA	(5Z,8Z,11Z,14Z,17Z)-eicosapentaeneoic acid		−50

Lipid-ukotvené proteiny



Inflammation

LPS signaling via toll-like receptor 4 (TLR4) activates NOS2 isoform.

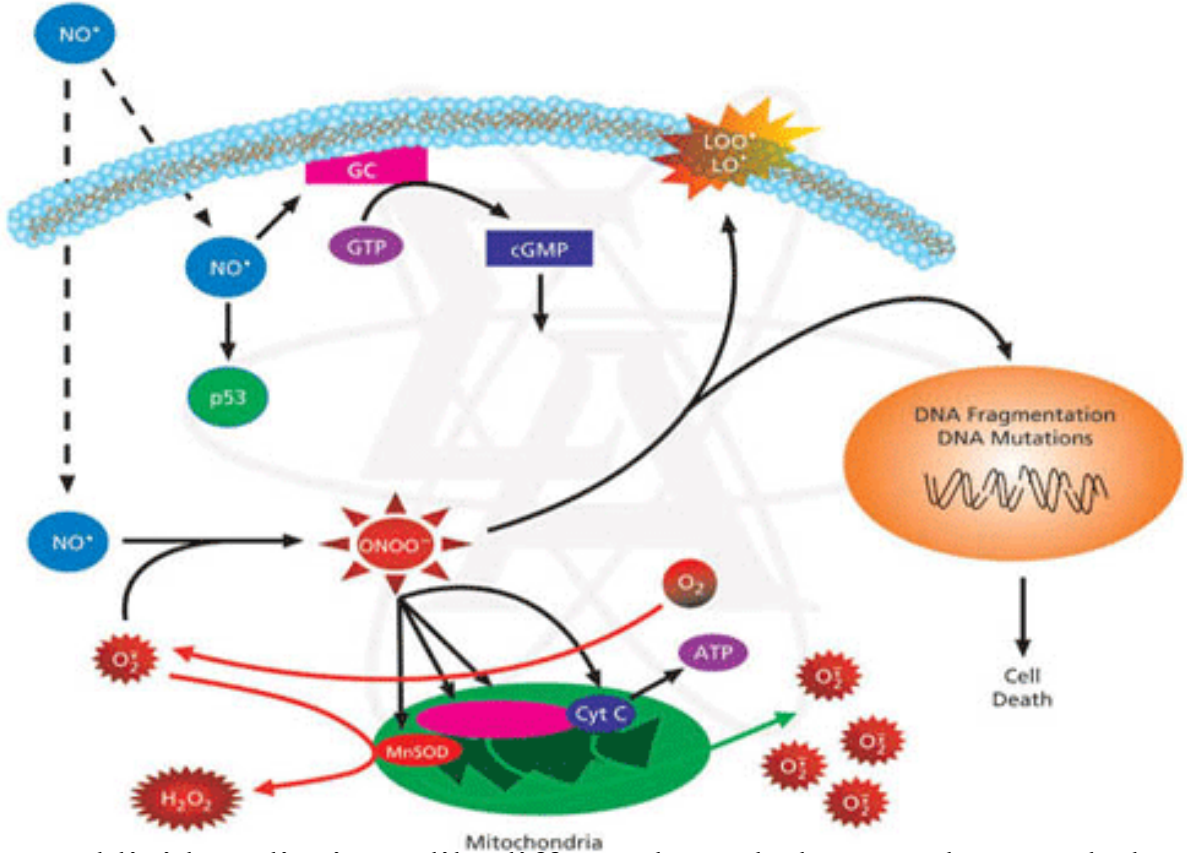


Oxygen free radicals

In addition to direct regulation of NO-synthases, NO availability is also dependent on the quantity of oxygen free radicals generated by cells surrounding NO-producer cell.

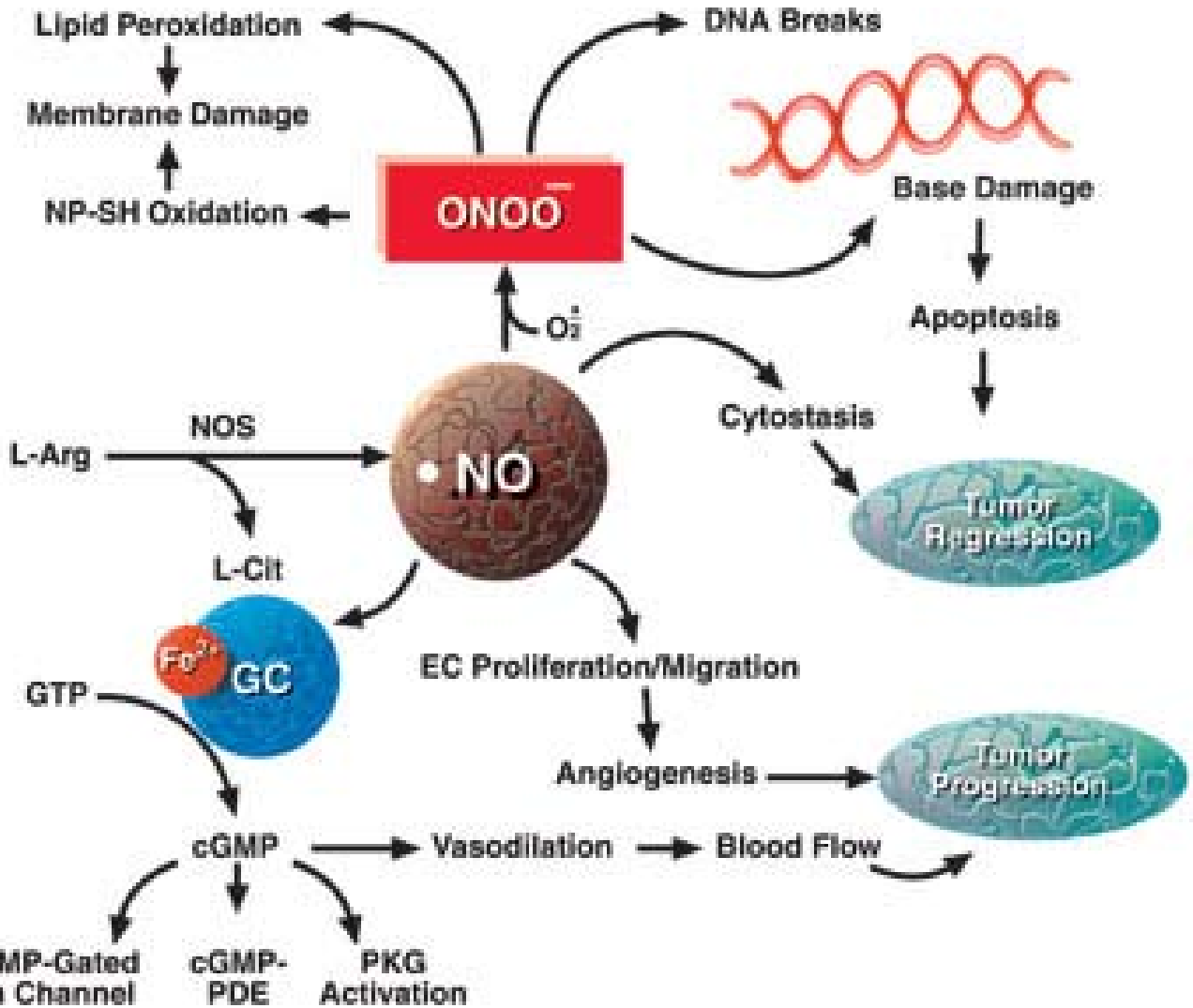
Nitric oxide synthases (eNOS and, especially, nNOS) also can produce superoxide under conditions of substrate (arginine) and/or tetrahydropteridine depletion, transferring electrons to oxygen instead of substrate (arginine). Whatever the origin of superoxide (eNOS, xanthine oxidase,...) this compound rapidly reacts with NO to form peroxynitrite.

In the intermediate range of cofactor concentration, one subunit of NOS can act as nitric oxide synthase and the other produce superoxide, acting as a peroxynitrite synthase as a whole



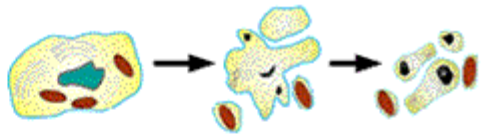
Nitric Oxide Metabolism

1. NO is soluble in both aqueous and lipid media, it readily diffuses through the cytoplasm and plasma membranes.
2. NO has effects on neuronal transmission.
3. In the vasculature, NO reacts with iron in the active site of the enzyme guanylyl cyclase (GC).
4. NO may also be involved in the regulation of protein activity through S-nitrosylation (the covalent attachment of a nitrogen monoxide group to the thiol side chain of cysteine).
5. In the extracellular milieu, NO reacts with oxygen and water to form nitrates and nitrites.
6. NO toxicity is linked to its ability to combine with superoxide anions (O^{2-}) to form peroxynitrite ($ONOO^-$), an oxidizing free radical that can cause DNA fragmentation and lipid oxidation.
7. In the mitochondria, $ONOO^-$ acts on the respiratory chain (I-IV) complex and manganese superoxide dismutase (MnSOD), to generate superoxide anions and hydrogen peroxide (H_2O_2), respectively.

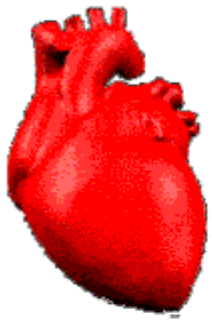


cGMP aktivuje specifickou kinázu (PKG), která fosforylací myosinu inhibuje kontrakci.

Parkinson's Disease



Programmed Cell Death



NO•

Antimicrobial Agent

Cardiovascular Disease

Impotence (Viagra®)