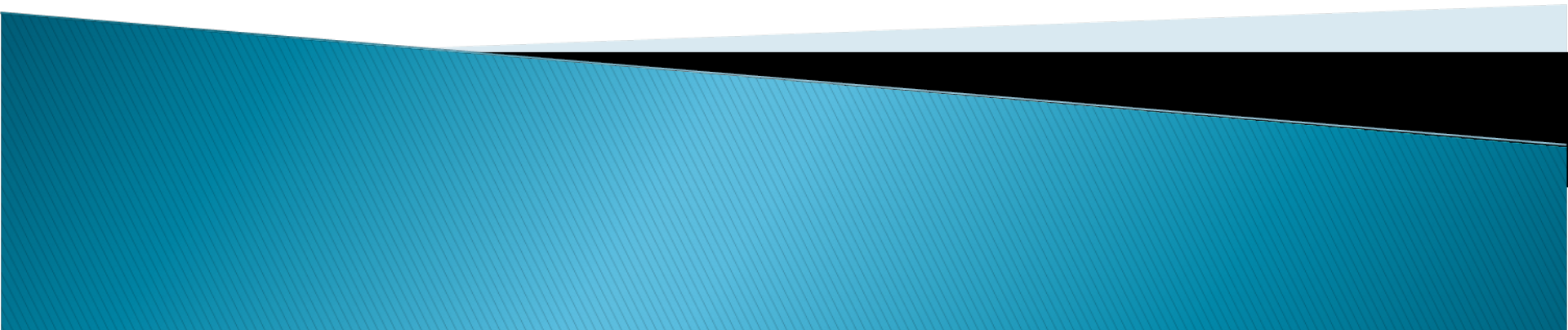


# Nitric Oxide in Biology

Chem 489

March 9, 2010

Rachel Chupik, Chris Costanzo



## MOLECULE OF THE YEAR



# NO News Is Good News

startlingly simple molecule unites neuroscience, physiology, and immunology and revises scientists' understanding of how cells communicate and defend themselves

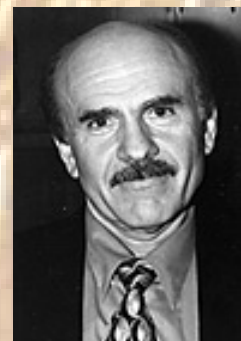
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## Various

## Biological Functions (Est. ~ 1987–1993)

- Vasodilation
- Prevention of Platelet Aggregation
- Central Nervous System (neurotransmission)
- Brain (memory/learning)
- Immune Systems (NO deficiency)
- Cytotoxicity

## 1998 Nobel Prize Physiology or Medicine



Robert Furchgott, Louis Ignarro, and Ferid Murad

# Nitric Oxide

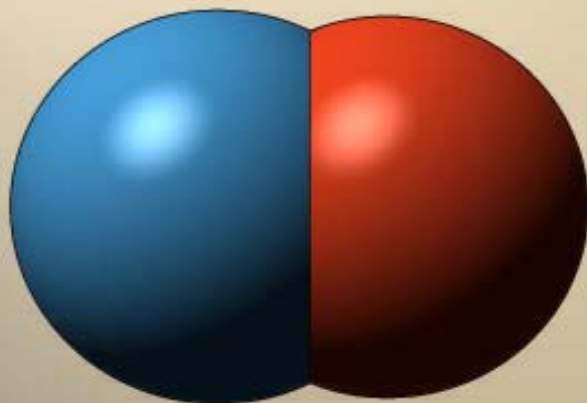
**Molecular formula**

NO

**Structural formula**

$\text{N}=\text{O}$

**Molecular models**



**Space filling**



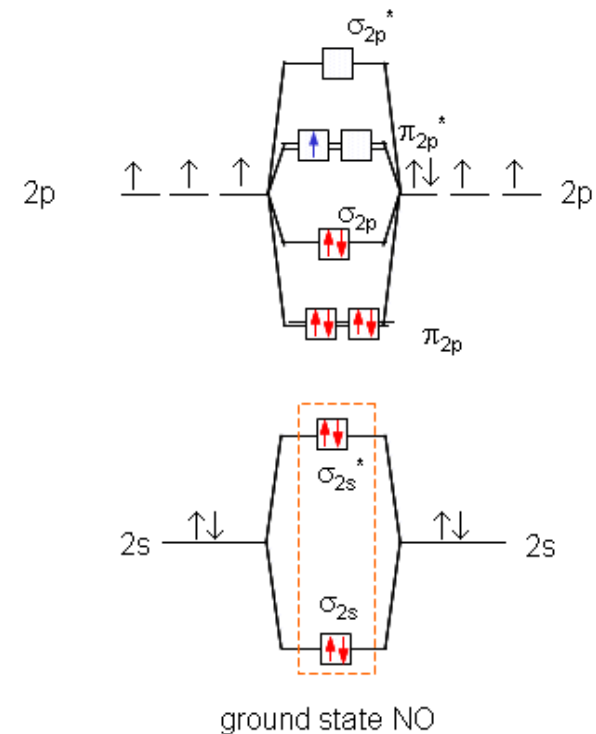
**Ball-and-stick**

**NITRIC OXIDE**

# Properties of NO (the molecule)

- ▶ Radical;  $S = 1/2$
- ▶ Bond order of 2.5
- ▶ Bond length 115 pm
- ▶ Linear
- ▶ Paramagnetic (ground state)
- ▶ Symmetry:  $C_{\infty v}$

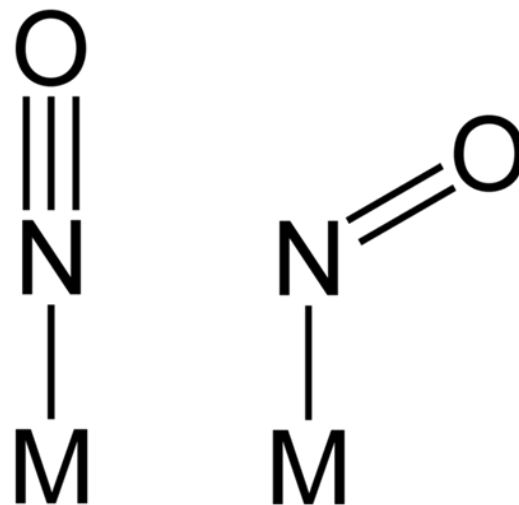
MOLECULAR ORBITALS NITRIC OXIDE - NO



# Properties of NO (the ligand)

- ▶ Forms complexes with all transition metals
- ▶  $\text{NO}^+$ 
  - Isoelectronic with CO
  - B.O. = 3
  - Binds linearly to metal (terminal linear most common)
  - M–N–O angle varies from 160 to 180 degrees
- ▶  $\text{NO}^-$ 
  - Isoelectronic with  $\text{O}_2$
  - B.O. = 2
  - Binds to metal in bent fashion

[http://en.wikipedia.org/wiki/Metal\\_nitrosyl](http://en.wikipedia.org/wiki/Metal_nitrosyl)



# History

## ▶ 1965 Barry Commoner Rat Experiment

Nitrite enhances the intensity of the abnormal electron spin resonance signal and inhibits the carcinogenicity of 2-acetylaminofluorene.

## ▶ Ferid Murad

- Discovered nitroglycerine released NO in the body

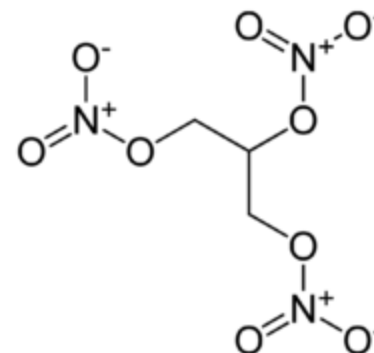
## ▶ M.L. Roussin–1858

- Roussin Salts
- reactions between nitroprusside ion,  $[\text{Fe}(\text{CN})_5\text{NO}]^{2-}$ , and sulfur, making it the first synthetic iron–sulfur cluster.

# History

- ▶ William Murrell (Discovery/Treatment 1858–1878)
  - experimented with the use of nitroglycerin to alleviate angina pectoris and reduce blood pressure
  - He would treat patients with small doses of nitroglycerin, but would call it Trinitrin or Glyceryl Trinitrate to not alarm the public that he was treating them with explosives.

◦ <http://www3.interscience.wiley.com/journal/119184830/abstract?CRETRY=1&SRETRY=0>

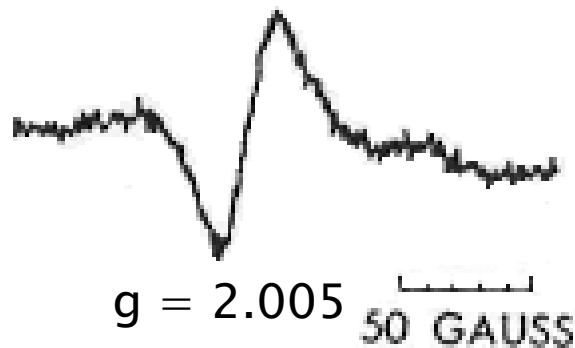


# Discovery of DNIC complexes in biology

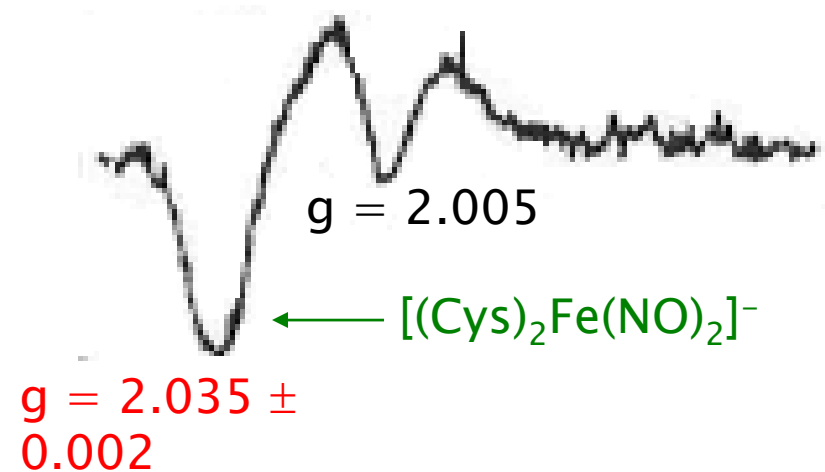
Barry Commoner, et al., Nature, Sept., 1965

“Change in Electron Spin Resonance Signals of Rat Liver during Chemical Carcinogenesis”

Healthy Rat Liver



Livers From Rats Fed Carcinogens



Chemicals:

*p*-dimethylaminoazobenzene, thioacetamide

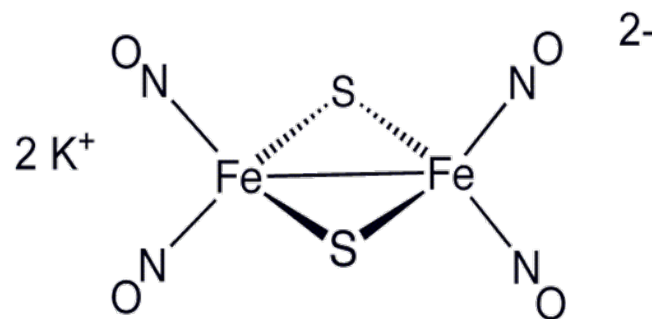


# History Cont.

## ▶ Roussin's Salts

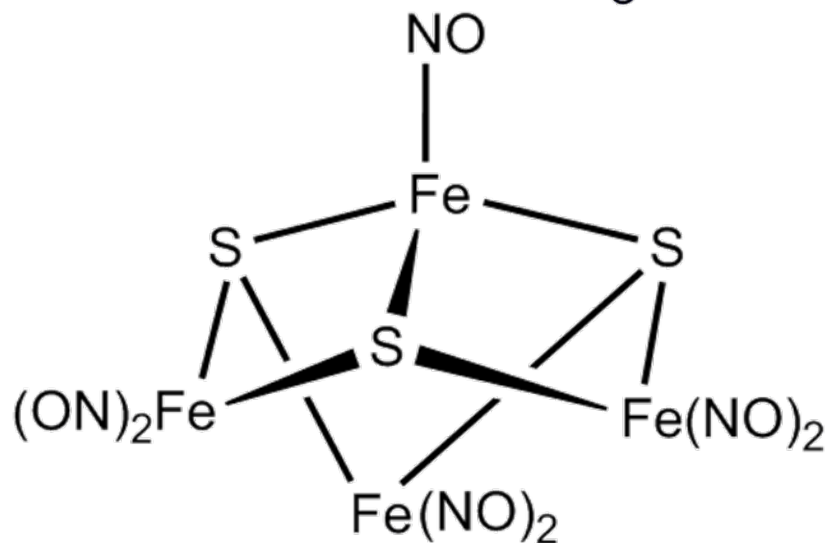
### ▶ Red salt:

- $D_{2h}$



### ▶ Black Salt:

- $C_{3v}$



# Roussin Salts

## ▶ Red Salt

- Today one common use of Roussin's red salt is of the ester derivative. This class of compounds is being investigated as a nitric oxide donor in biology and medicine. Due to its relatively low toxicity and good stability Roussin's red salt can act as a possible chemotherapeutic. Photolysis of the compound induces the release of NO, thereby sensitizing target cells to exposure to radiation

## ▶ Roussin's Black Salt

Nitric Oxide donor. The nitric oxide donated by Roussin's Black Salt has proven to be toxic to some melanoma cancer cells. Also, Roussin's Black Salt demonstrates antibacterial activity in some food processing applications.

[http://en.wikipedia.org/wiki/Roussin's\\_black\\_salt](http://en.wikipedia.org/wiki/Roussin's_black_salt)

[http://en.wikipedia.org/wiki/Roussin's\\_red\\_salt](http://en.wikipedia.org/wiki/Roussin's_red_salt)

# NO in Biology

- ▶ Signaling Agent and Messenger
  - Diffuses freely across cell membranes
  - Consumed quickly near where it is synthesized
  - Relaxes smooth muscle which allows for greater blood flow, regulates gastric contractions, and other benefits
- ▶ How it's found in the body
  - Hemoglobin
  - Myoglobin
  - S-nitrosothiols

Unclear how NO reaches body part if not synthesized there

- Possibly through RSNO, DNIC or porphyrin iron

# Signaling Mechanism

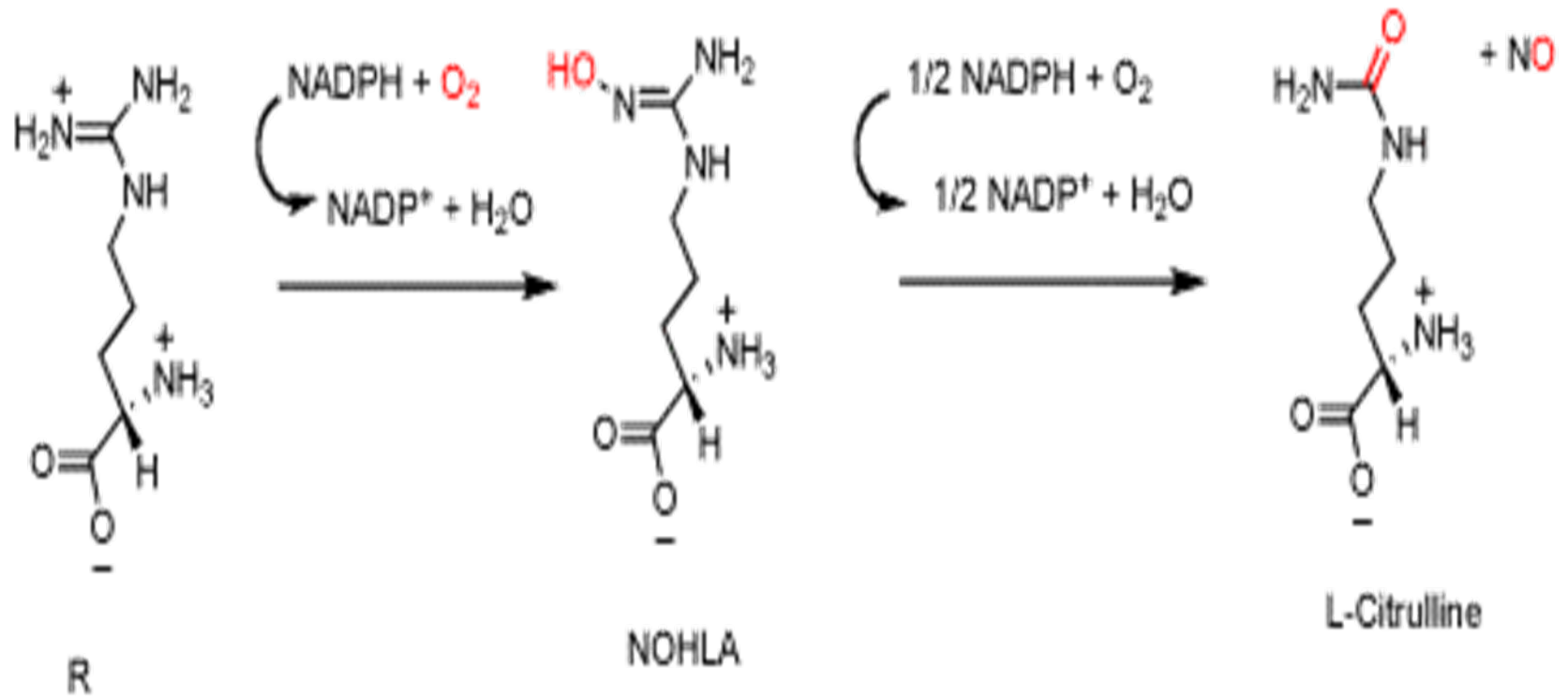
- ▶ The signaling functions of NO begin with its binding to protein receptors on or in the cell. The binding sites can be either:
  - A metal ion in the protein
  - One of its S atoms (e.g. on cysteine)
  - In either case, binding triggers an allosteric change in the protein which, in turn, triggers the formation of a "second messenger" within the cell

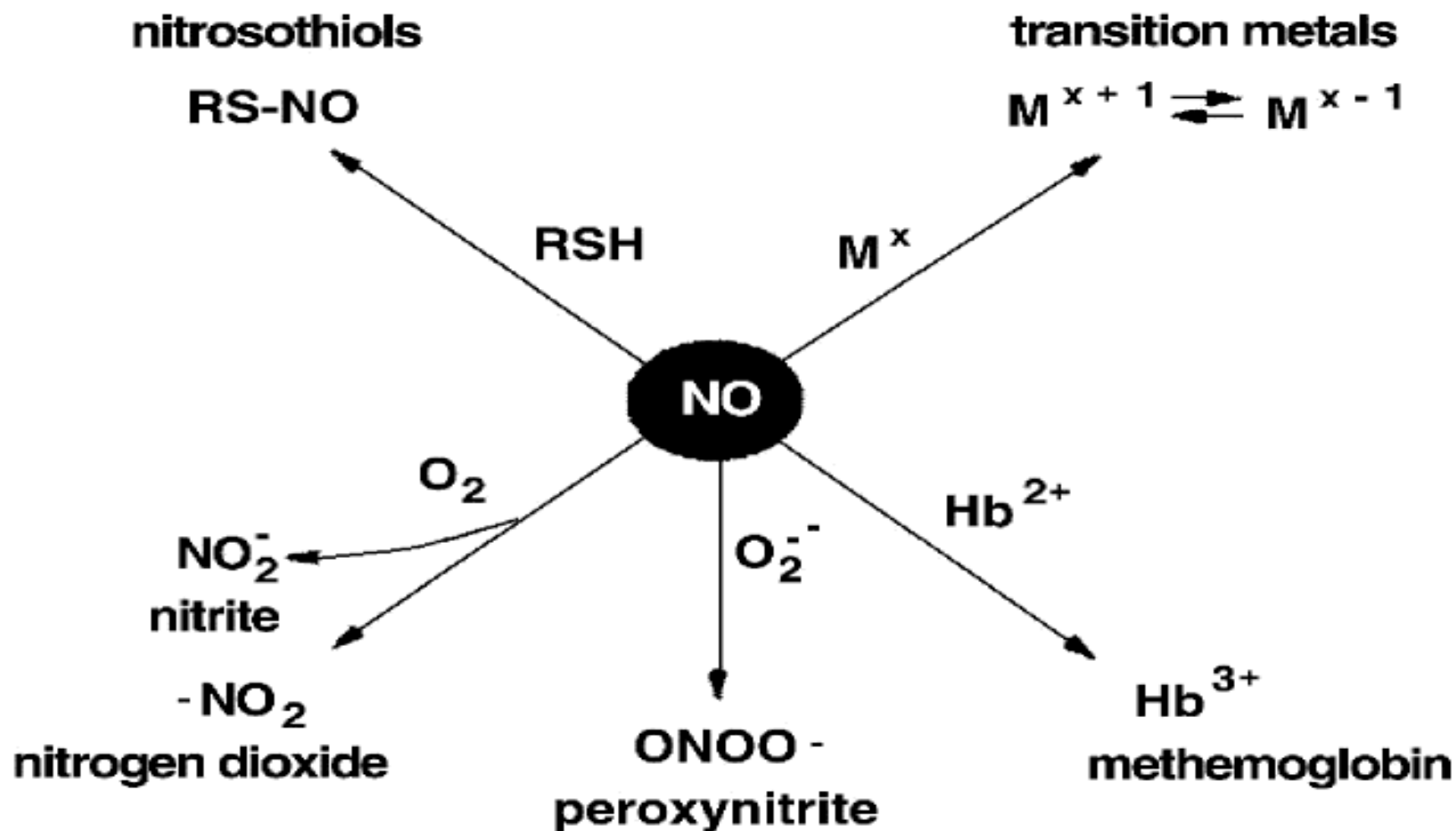
- <http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/N/NO.html>

# NO as Messenger in Biology

- ▶ Neuronal NOS (nNOS)
  - Produces NO in nervous tissue
  - Cell communication
- ▶ Inducible NOS (iNOS)
  - Immune defense against pathogens
- ▶ Endothelial NOS (eNOS)
  - Generates NO in blood vessels
- ▶ Bacterial NOS (bNOS)
  - Defends against oxidative stress, antibiotics, immune attack

# NO Synthase in Body

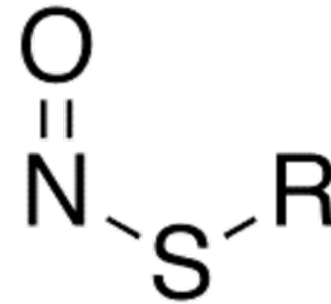




Source: Curr Opin Pul Med © 2004 Lippincott Williams &amp; Wilkins

► [www.chestjournal.org/.../F1.expansion?ck=nck](http://www.chestjournal.org/.../F1.expansion?ck=nck)

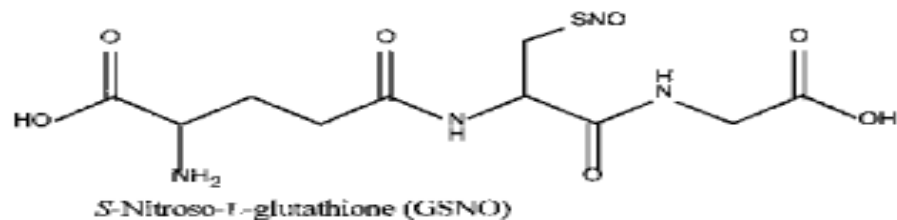
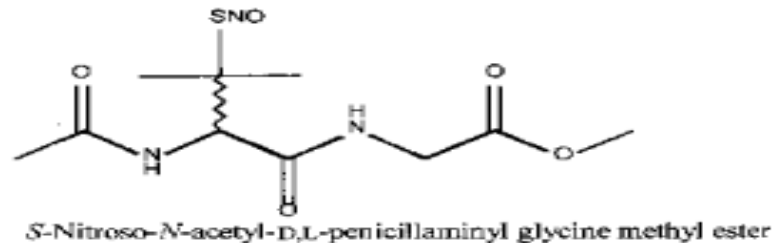
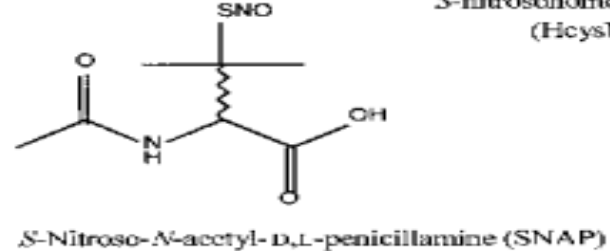
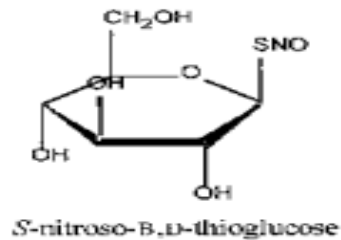
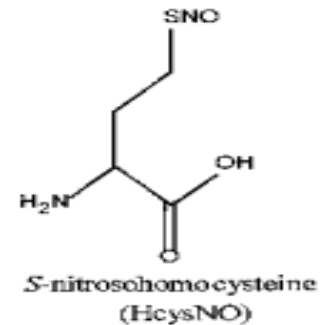
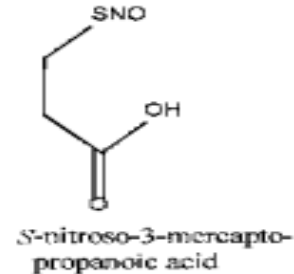
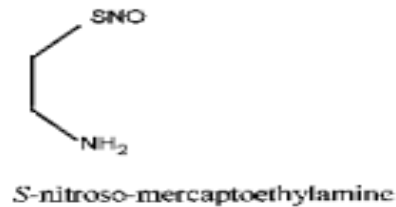
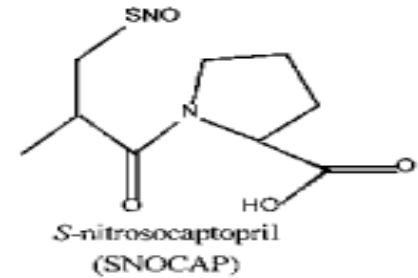
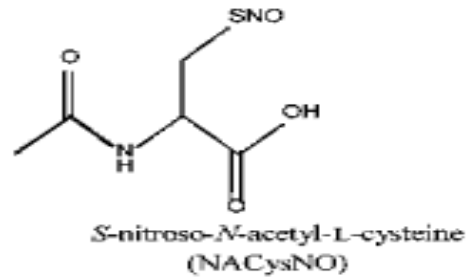
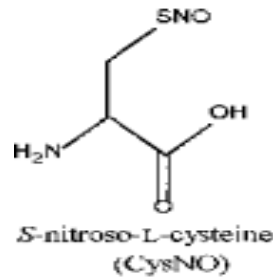
# Sulfur-NO



- ▶ S-nitrosothiols
  - $2\text{RSNO} \rightarrow \text{RSSR} + 2\text{NO}$
- ▶ Play important role in NO-mediated biological events
  - Release the Nitronium Ion to signal vasodilation
    - Red blood cells release S-nitrosothiols into the bloodstream under low-oxygen conditions, causing the blood vessels to dilate



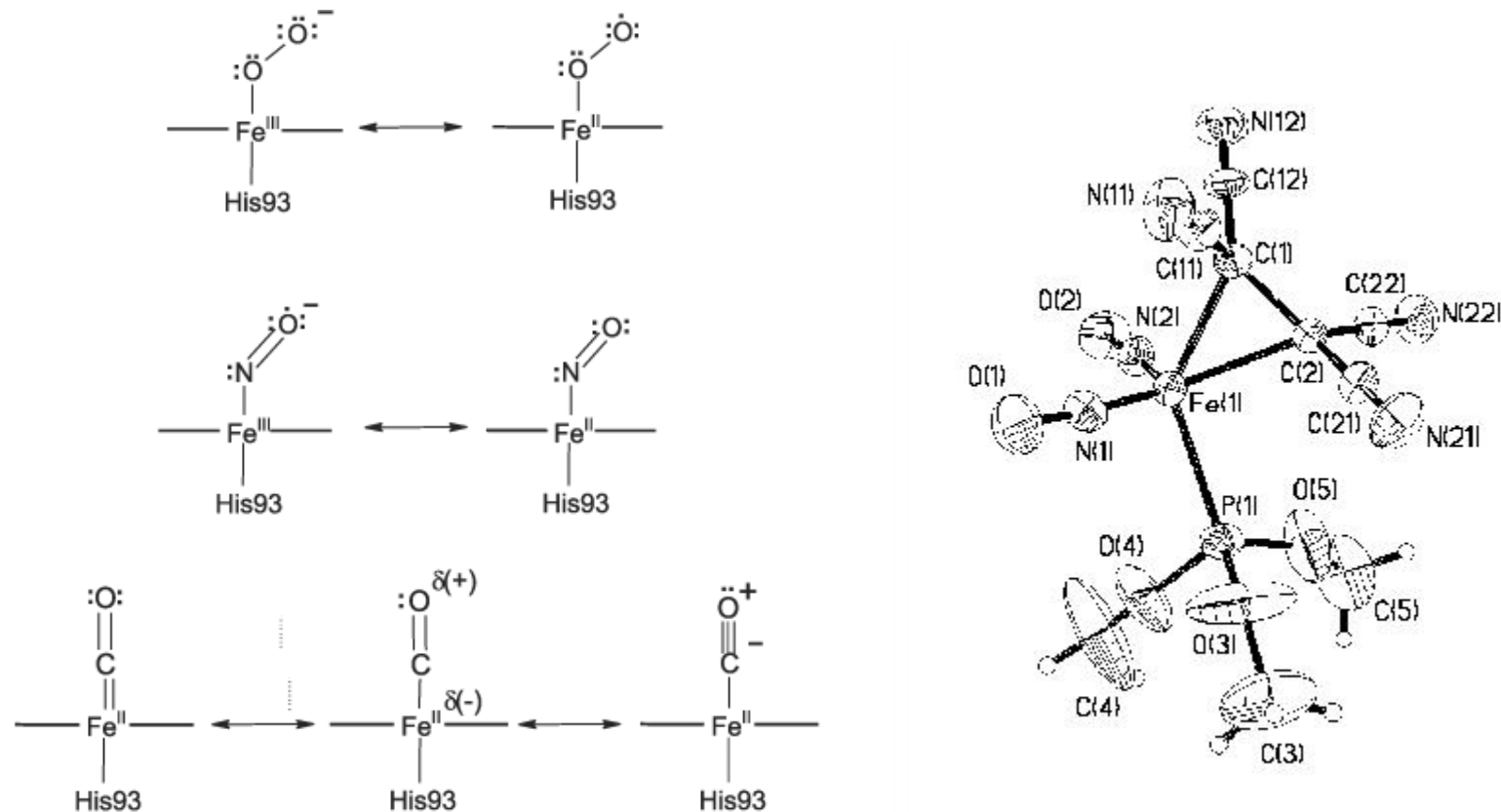
# Synthetic and naturally occurring S-nitrosothiols



# NO as ligand to Iron

- ▶ Three types of complexes
  - Iron porphyrin nitrosyls
  - Dinitrosyl Complexes
  - Iron sulfur cluster nitrosyls

# Porphyrin and Dinitrosyl NO



**Figure 1.** Fe-ligand bonding geometry and simplified electronic distribution for ferrous myoglobin complexes of NO, O<sub>2</sub> and CO. Modified from refs. 8 and 13

# Iron Porphyrin Nitrosyls

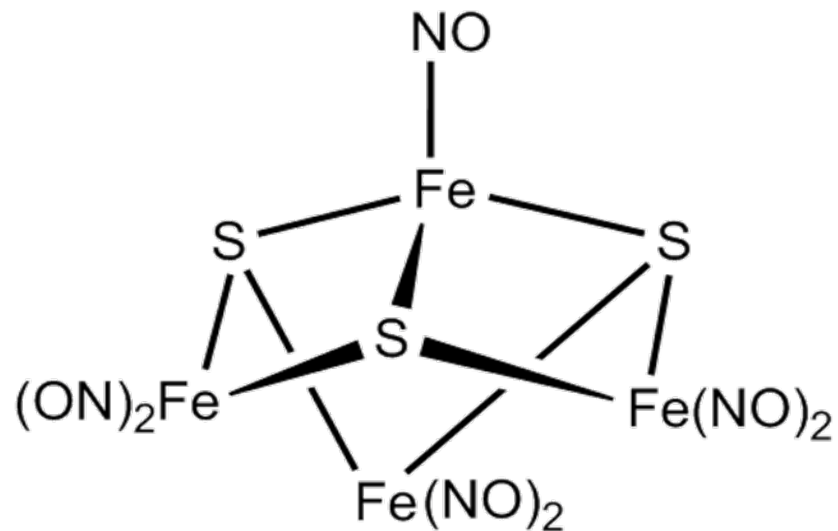
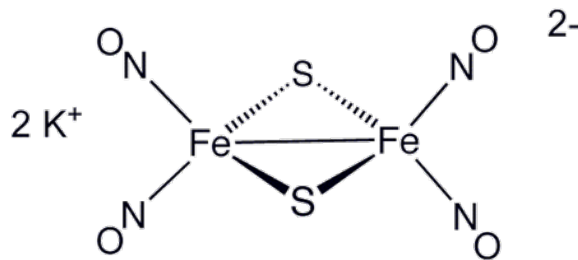
- ▶ Physiological effects of NO mediated through enzyme receptor soluble guanylyl cyclase (sGC)
  - ▶ sGC1: 6-coordinate heme with 2 histidines
  - ▶ sGC2:
    - High spin; 5-coordinate
    - Square pyramidal with 1 histidine
  - ▶ Myoglobin
    - Efficient NO scavenger (NO recombines with heme-Fe(II) within first 5ps after photolysis)
  - ▶ Hemoglobin
    - S-nitroso-hemoglobin (SNOHb) regulates blood flow and oxygenation
- ▶ Bent Fe-N=O

# Dinitrosyl Complexes

- ▶ Formed during reaction of Fe(II) with NO in presence of low MW thiols, aminoacids, peptides or proteins
- ▶ With low weight thiols, can inter convert between paramagnetic and diamagnetic if Fe<sup>2+</sup>: thiol ratio correct

# Iron Sulfur Cluster Nitrosyls

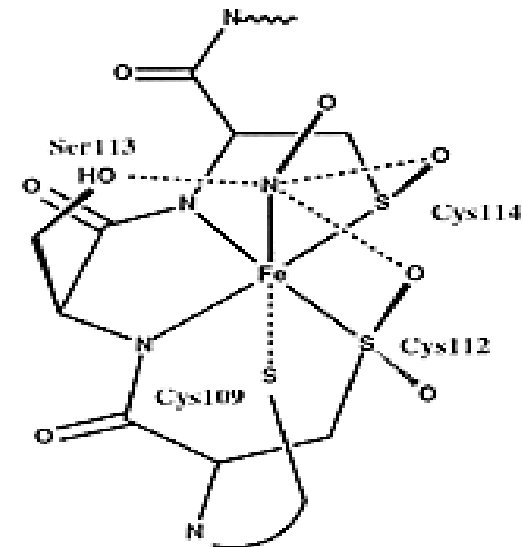
- ▶ Generated from NO interactions with Fe–S cluster moieties of enzymes
- ▶ In presence of excess NO
- ▶ Examples: Roussins salts



# Functions of NO as ligand to Fe

## ► Nitrile hydratase

- Mononuclear iron catalyzed hydration
  - $\text{R}-\text{C}\equiv\text{N} + \text{H}_2\text{O} \rightarrow \text{R}-\text{C}(\text{O})\text{NH}_2$
- Composed of two subunits ( $\alpha$  and  $\beta$ )
- Exist as  $\alpha\beta$  dimers or  $\alpha_2\beta_2$  tetramers
- Bind one metal atom per  $\alpha\beta$  unit



Nitrile Hydratase: isolated as NO complex inactive form

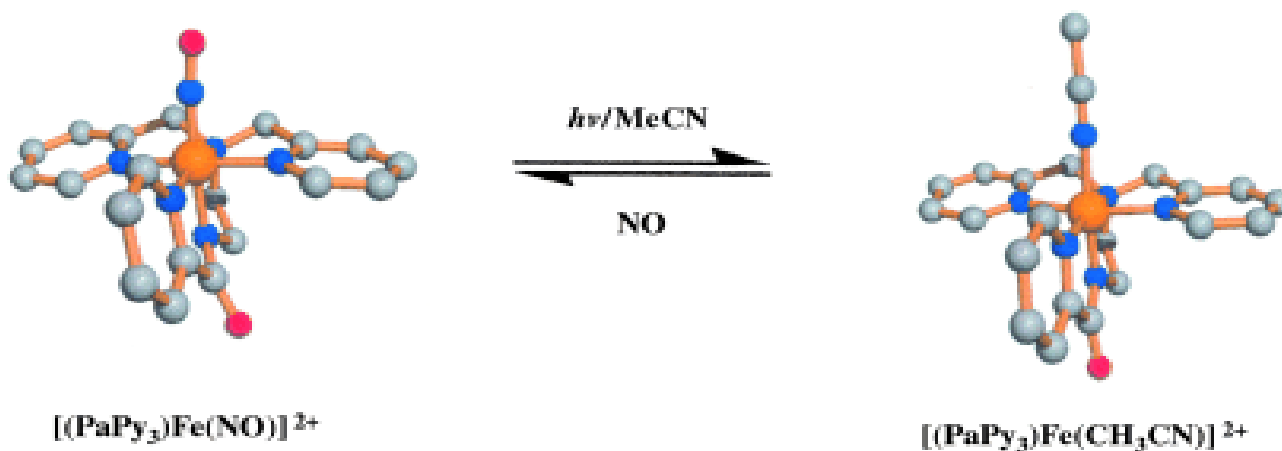
## ► First iron nitrosyl complex prepared as far back as 1850 (Playfair)

- $[\text{Fe}(\text{CN})_6]^{4-} + \text{NO}^- + \text{H}_2\text{O} \rightarrow [\text{Fe}(\text{CN})_5\text{NO}]^{2-} + \text{CN}^- + 2\text{OH}^-$

- [http://en.wikipedia.org/wiki/Nitrile\\_hydratase](http://en.wikipedia.org/wiki/Nitrile_hydratase); Metal-NO complexes: Structures, Syntheses, Properties and NO-releasing Mechanisms Tara P. Dasgupta, Danielle V. Aquart

# Nitrile Hydratase

- ▶ NO version inactive
- ▶ NO photochemically removed to form open site for iron to bind nitrile



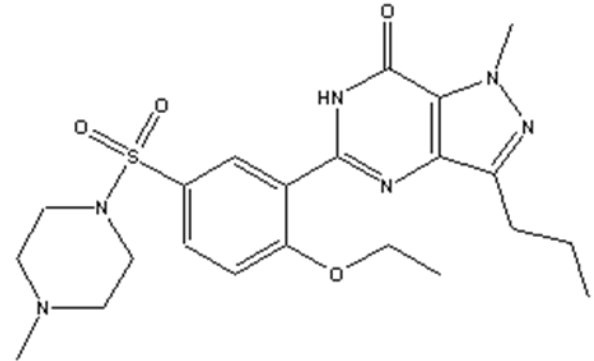
- ▶ <http://www3.interscience.wiley.com/journal/119184830/abstract?CRETRY=1&SRETRY=0>



# Products

- ▶ Three prescription drugs  
sildenafil (Viagra®)  
vardenafil (Levitra®)  
tadalafil (Cialis®)

Enhance the effects of NO by inhibiting the enzyme that normally breaks down cGMP



## Nitroglycerine

Lowers blood pressure by dialating blood vessels.

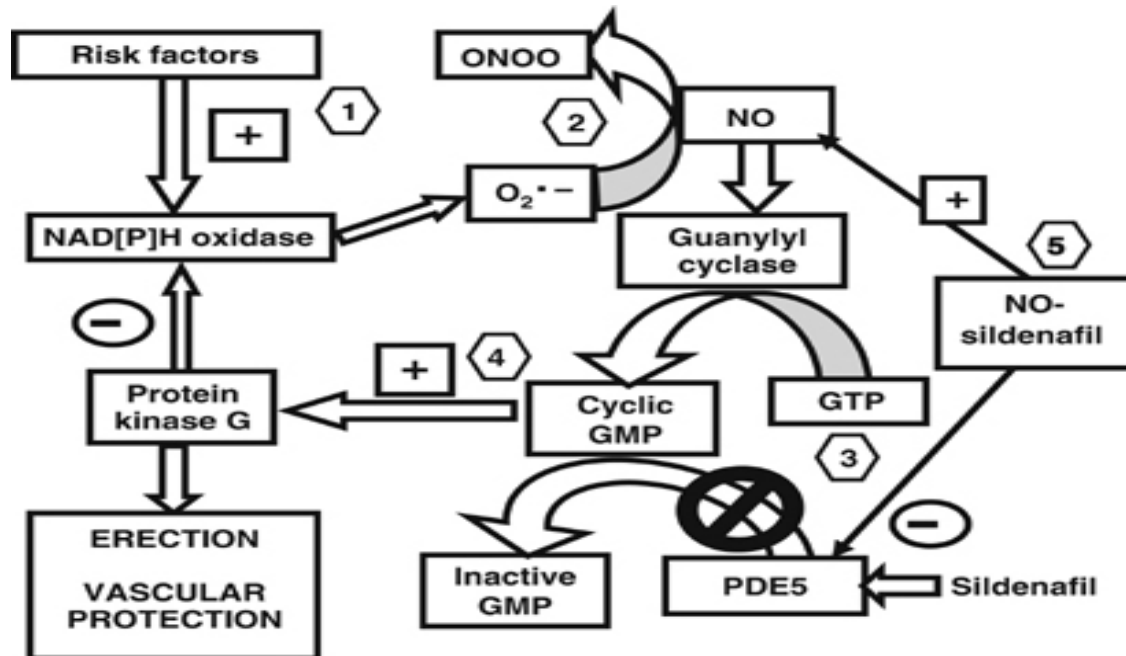
Smooth muscle tissue is relaxed along vessel walls

## Athletic Supplements

Contain high amounts of Arginine to produce NO from the NO synthase reaction

# Sildenafil

- ▶ Marketed by Pfizer as Viagra
- ▶ Inhibits degradation of cGMP by PDE5
  - Does not actually contain NO



- [www.nature.com/.../v19/n3/fig\\_tab/3901523f4.html](http://www.nature.com/.../v19/n3/fig_tab/3901523f4.html)