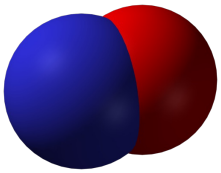
Nitric Oxide

(.N=O)



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https://physiology.cuni.cz
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UNIVERZITA KARLOVA
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Why is NO interesting?

- Small anorg. molecule, large biol. importance
  - MW = 30 (O_2 = 31, Ca^{2+} = 40)
- Participates in the function of all main organ systems
- 2 faces: signalling x toxicity

- From the basic discovery to fundamental advances in clinical practice in a few years

History: 1620

NO first prepared:

Jan Baptista van Helmont

(Flemish, 1577-1644)

$Cu + HNO_3 \rightarrow Cu^{2+} + NO + H_2O$

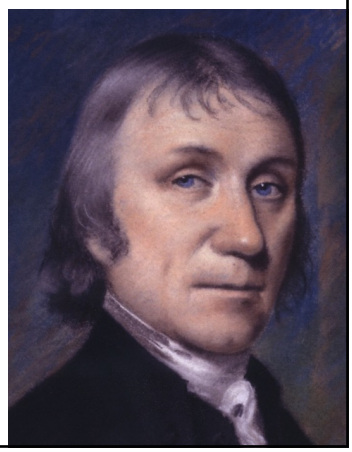
(i.e. earlier than e.g. oxygen - 1774)

1772

Chemical characterization:

Joseph Priestley

(the discoverer of oxygen)



1800

Toxicity:

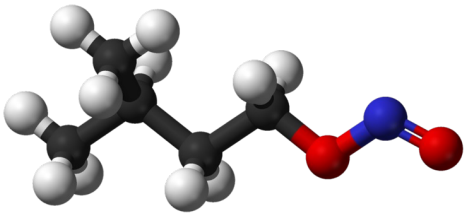
Sir Humphry Davy

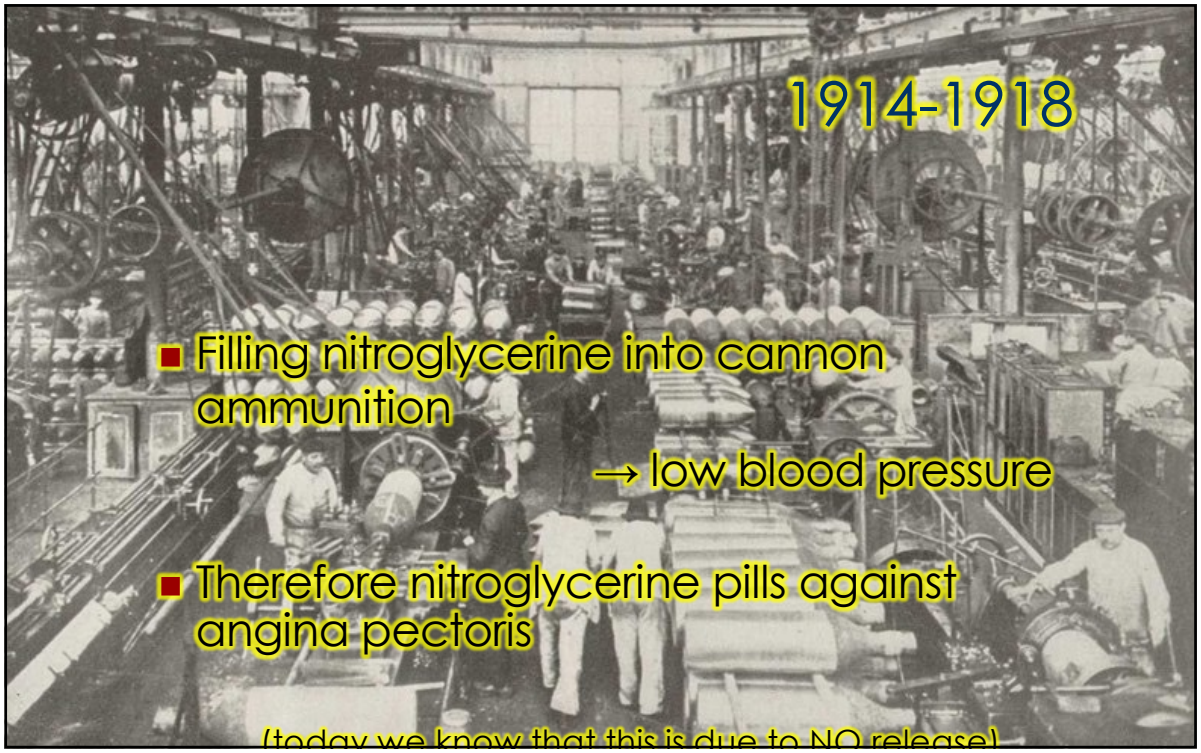
(almost died after inhaling NO)

1867

Amylnitrite ($C_5H_{11}ONO$) lowers blood pressure in hypertension

(today we know that this is due to NO release)





1977

NO activates guanylate cyclase, thus increasing intracellular cGMP concentration:

Ferid Murad

1980

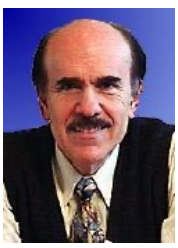
Endothelium-derived relaxing factor (EDRF):

Robert Furchgott

1987

Eukaryotic cells can make NO:

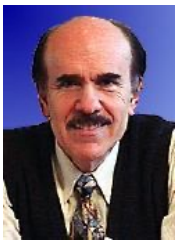
Louis Ignarro, Salvador Moncada



1998

Nobel Prize for Physiology and Medicine

"For key discoveries regarding NO as a signal molecule in the cardiovascular system"



NO chemistry

- NO is a gas (colorless)
  (liquidifies at -152oC, solidifies at -164°C)
- NO is a radical
  - i.e. odd number of valency electrons
  - NO has 11 (N_2 has 10; O_2 has 12)
- Direct synthesis from N_2 and O_2 only under specific conditions (e.g. lightning, combustion engines, power plants)

NO solubility

- Low solubility in water
  - ~1.7 mmol/l at 25°C
  - i.e. similar to O_2 or N_2
- Lipophility → easy passage through membranes

Spontaneous decay of NO

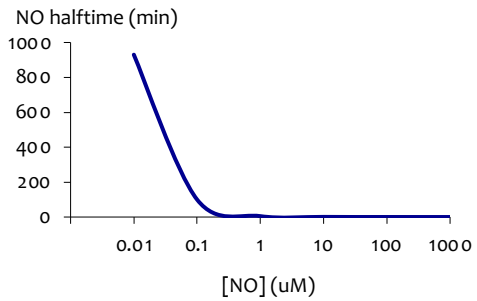
- Only under high pressure
- Gradual conversion to toxic NO_2
  (! storage in pressurized cylinders !)

Oxidation of NO

- In the presence of O_2:

$$2\ NO + O_2 \rightarrow 2\ NO_2$$

- NO_2 (nitrogen dioxide) is a toxic radical (brown gas)
- Fast (several sec), if there is lots of NO and O_2
- Slow, if NO is scarce
  - that is usually the case in tissues (NO < 10 μM, NO half time ~ 500 sec)



Oxidation of NO

- ~ 200x faster in solution than in gas phase
- End products in solution: nitrites (NO_2^-), resp. HNO_2
- Proceeds to nitrates (NO_3^-) only in the presence of hemoproteins

Physiological role of nitrites (NO_2^-)

- "Storage" of NO in blood and tissues
- Easy reduction to bioactive NO
  - non-enzymaticaly
  - XORs, NOS, cytochromes, deoxyhemoglobin, deoxymyoglobin
- ↑NO_2^- reduction to NO at low O_2 (helps hypoxic vasodilation)

Nitrates (NO_3^-) in food

- rich in leafy green vegetables (and some roots)
- reduced to NO_2^- by commensal bacteria on tongue
- NO_2^- further reduced to NO in the stomach by low pH → kills almost all bacteria swallowed with food
- similarly protection of skin from fungi: NO_3^- in sweat reduced to NO_2^- by commensal microorganisms on skin and further to NO by the slightly acidic skin surface
- NO_3^- contribute to + effects of vegetables (prevention of cardiovascular diseases and DM type 2)

Reaction of NO with superoxide

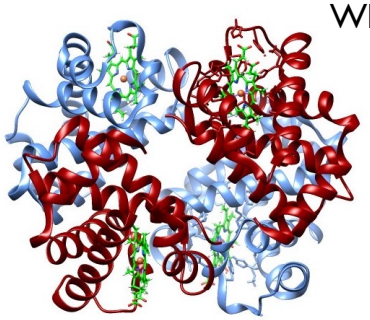
- O_2^- is a reactive oxygen radical
  - some is formed in respiratory chain
  - high production at inflammation sites (NADPH oxidase)
- O_2^- & NO form very quickly peroxynitrite:

$$NO + O_2^- \rightarrow OONO^-$$

- $OONO^-$ is not a radical, but is highly reactive (> O_2^-) & cytotoxic (also nitrosylates) survives longer in biological systems

NO inactivation by hemoglobin

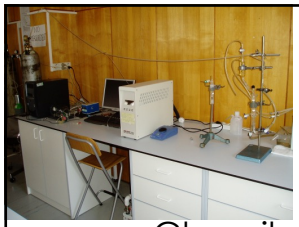
- NO has a high affinity to heme
- Fast inactivation of NO by oxidation with Fe of oxyHb yielding NO_3^-



nitrosoHb --> metHb -->
Hb reductase --> oxyHb

S-nitrosylation of proteins

- reversible binding of NO groups to sulfhydryl (-S-H) groups of proteins (posttranslational modification)
- affects
  - receptors coupled with G proteins
  - mitochondrial metabolism
  - $[Ca^{2+}]_i$
  - cell defense against oxidative stress & apoptosis



Measuring NO

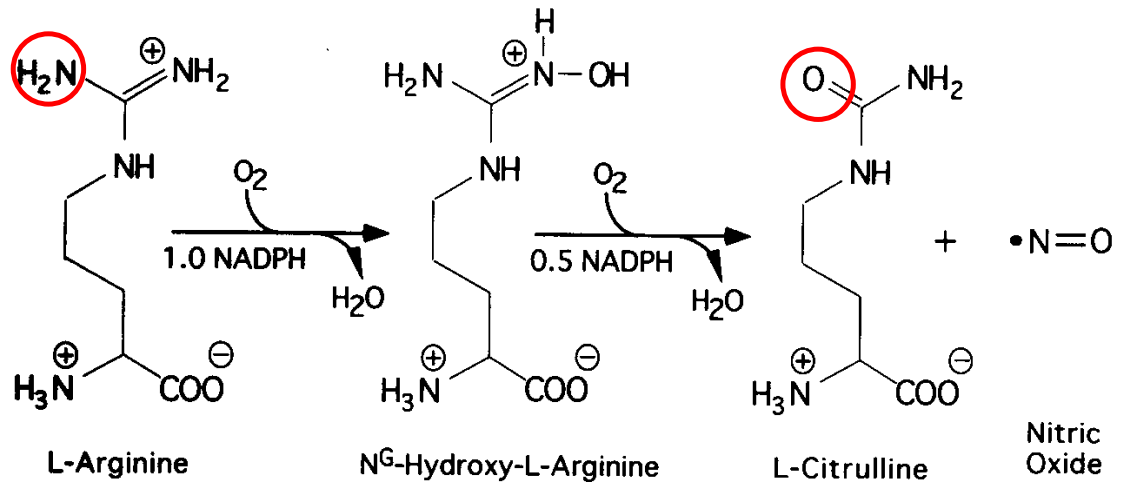
- Chemiluminescence ($NO + O_3 \rightarrow NO_2^* + O_2 \rightarrow NO_2 + hv$)
  - gas phase
  - liquid phase (stripping)
  - NO oxidation products (reducing chamber)
- Elektroanalysis (amperometry) - NO reacts with electrode → Δ current of voltage
- Spin trap: NO + Fe-dithiocarbamate complexes, then detection of mono-nitrosyl-Fe complexes by electron paramagnetic resonance (EPR)
- Fluorescence indicators (4,5-diaminofluorescein - DAF-2): intracellular measuremens

NO biosynthesis

- 5 electron oxidation of terminal guanidino nitrogen of L-arginine by molecular oxygen

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

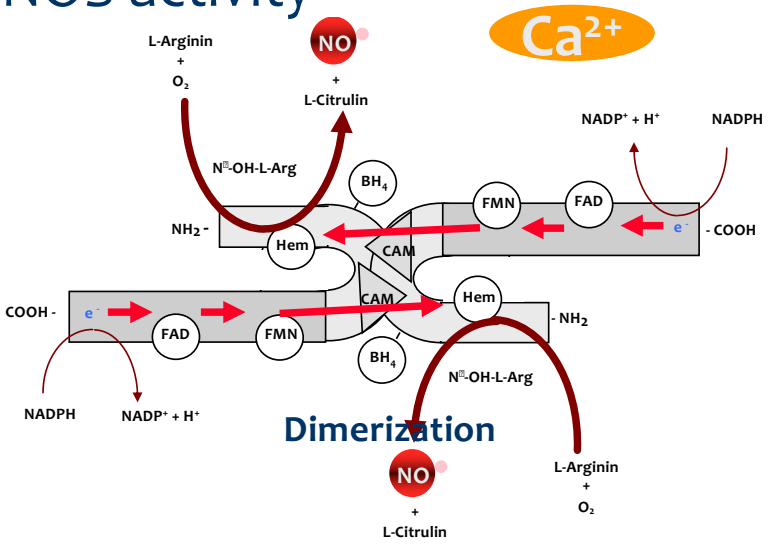
- Stereospecificity
- The whole reaction is catalyzed by a single enzyme, **NO synthase** (NOS, EC 1.14.13.39)



NO synthases

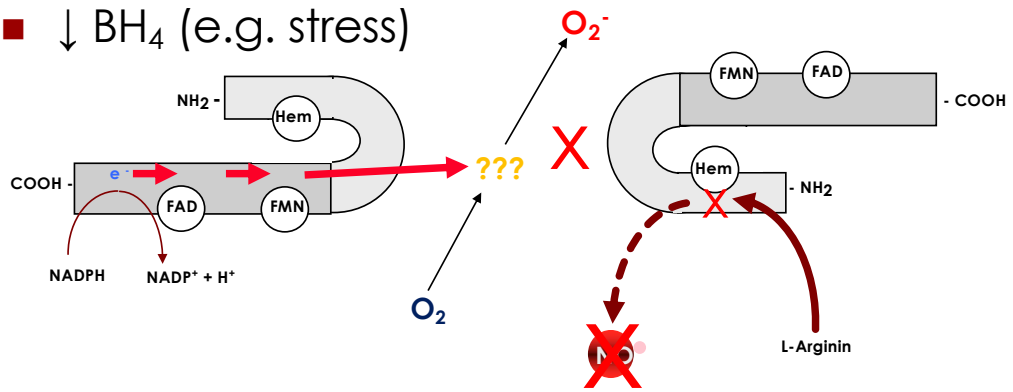
- 3 isoforms: I, II, III
  - all contain heme in the active center
  - active as homodimers
  - required cofactors:
    - NADPH
    - 6(R)-5,6,7,8-tetrahydrobiopterine
    - FAD
    - FMN
    - calmodulin

NOS activity

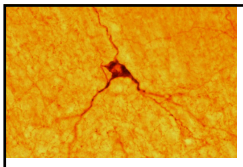


NOS uncoupling

- ↓ L-arg
- ↓ BH_4 (e.g. stress)

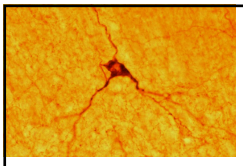


- NADPH consumption NADPH & O_2 reduction detached from NO production
- ↓ NO production, ↑O_2^- production
- one of the main mechanisms of endothelial dysfunction



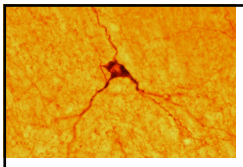
NOS I

- nNOS (neuronal)
- ~160 kDa
- Gene on human chromosome 12
- Requires Ca^{2+}
  (essential for calmodulin binding)
- Dissolved in cytosol



NOS I

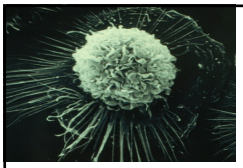
- Constitutively expressed:
  - central and peripheral neurons
  - some epithelial and vascular smooth muscle cells
  - skeletal muscle
- Regulation of activity:
  - Ca^{2+}
  - ser/tyr phosphorylation
  - NO (feedback inhibition)



NOS I

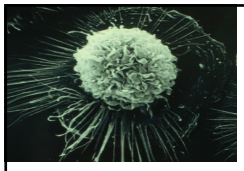
Main function:

- neurotransmission
- neuromodulation



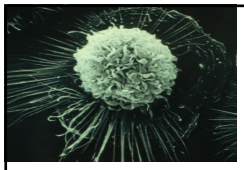
NOS II

- iNOS (inducible)
- ~130 kDa
- Gene on human chromosome 17
- Does not need Ca^{2+}
  (binds calmodulin permanently without Ca^{2+})
- Dissolved in cytosol



NOS II

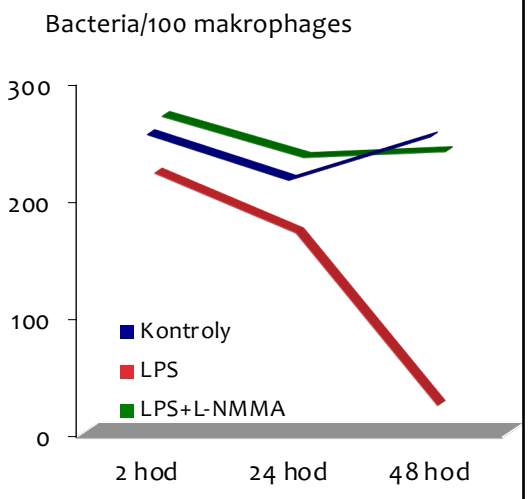
- Expression is inducible (cytokines,...):
  - macrophages
  - glial cells, hepatocytes
  - endothelium, epithelium
  - cardiac myocytes, smooth muscle,...
- Regulation of activity:
  - induction of expression
  - NO (feedback inhibition)

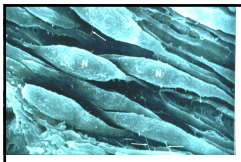


NOS II

Main function:

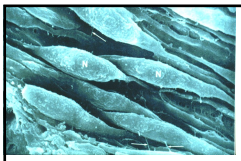
- fighting infection
  - bacteria
    (even those otherwise difficult to kill - e.g. Mycobacterium tuberculosis)
  - fungi
  - parasites
  - tumors
  - inhibits viral replication
- killing tumors





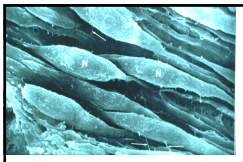
NOS III

- eNOS (endothelial)
- ~133 kDa
- Gene on human chromosome 7
- Requires Ca^{2+}
  (essential for calmodulin binding)
- Bound to cell mebrane (caveolae)



NOS III

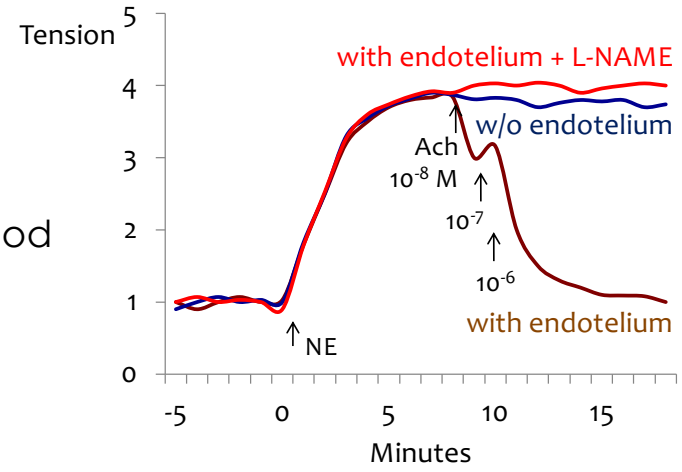
- Constitutively expressed:
  - endothelium
  - pulmonary and renal epithelium; thrombocytes
  - cardiac myocytes
  - hippocampus
- Regulation of activity:
  - Ca^{2+}
  - ser/tyr phosphorylation
  - modulation of expression
  - inhibition by S-nitrosylation (thiol groups of cysteins)



NOS III

Main function:

- vascular tone regulation
- regulation of blood supply to organs



Mitochondrial NOS

- Similar to NOS I
- Importance unknown

NO effects on target tissues

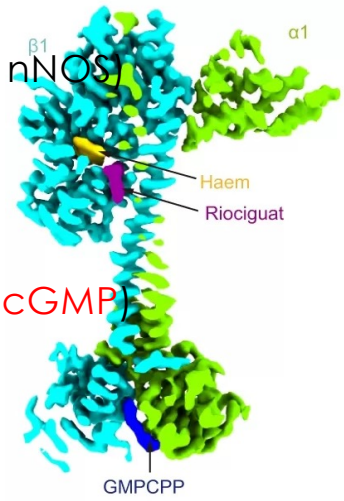
1. Cytotoxicity
2. cGMP

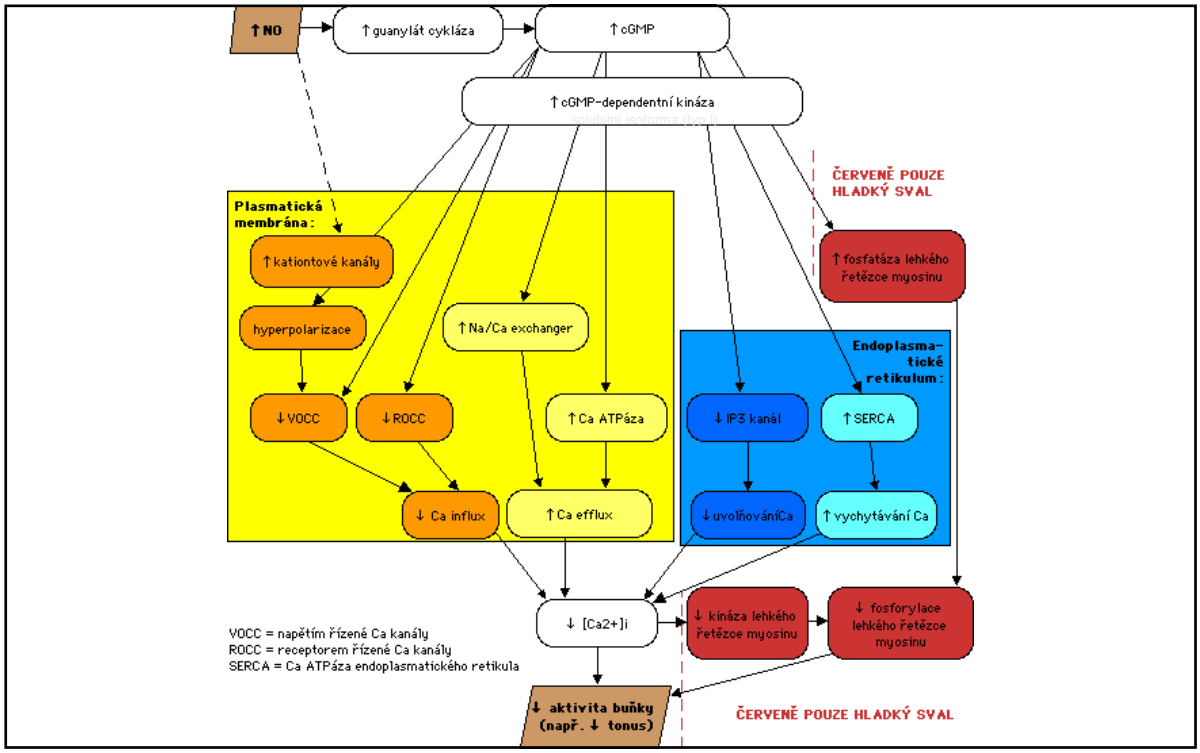
Effects of NO on target tissues

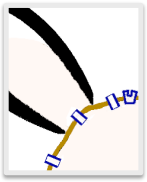
1. Cytotoxicity:
   - at high NO concentrations (iNOS)
   - damage to proteins, DNA, lipids
   - oxidation (O_2, O_2^-)
     —> reactive, toxic products (NO_2, $ONOO^-$)
   - inhibition of respiration
   - fights infection and tumors

Effects of NO on target tissues

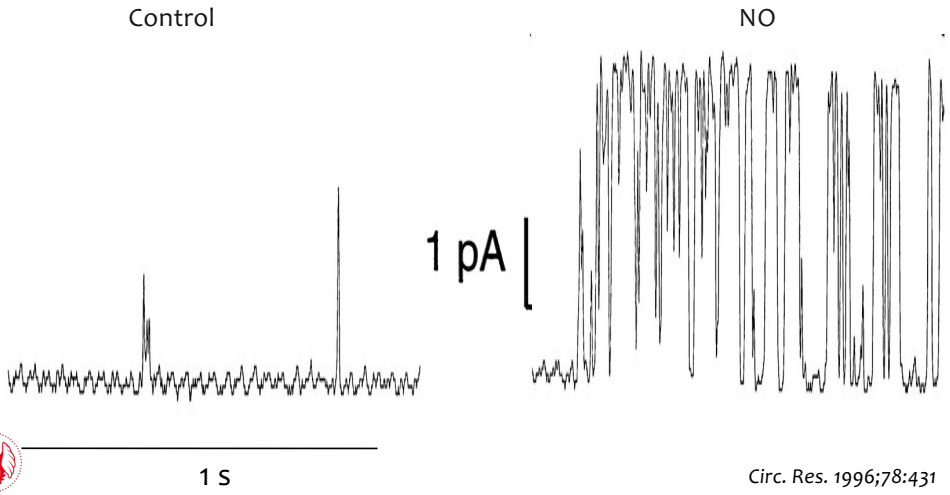
2. Via cGMP:
   - At lower NO concentrations (eNOS, nNOS)
     Oxidation slow
   - Binding of NO to the heme of the soluble isoform of guanylate cyclase prevails
   - ↑ guanosine-3',5' monophosphate (cGMP)
   - cGMP activates cGMP-dependent protein kinase (G-kinase)







NO activates K_{Ca} channels



Circ. Res. 1996;78:431

Fate of cGMP

cGMP inactivation:

Phosphodiesterases of cyclic nucleotides

- particularly type V.
- produce 5'-GMP

Pharmacology of NO

- NO donors
  (nitroglycerin, nitroprusside, NOates)
- NOS inhibitors
  (L-NMMA, L-NAME, aminoguadinine, 7-NINA, ADMA)
- eNOS activators
  (endothelium-dependent vasodilators)
- phosphodiesterase inhibitors
  (sildenafil, zaprinast)

Functions of NO: Neurotransmission

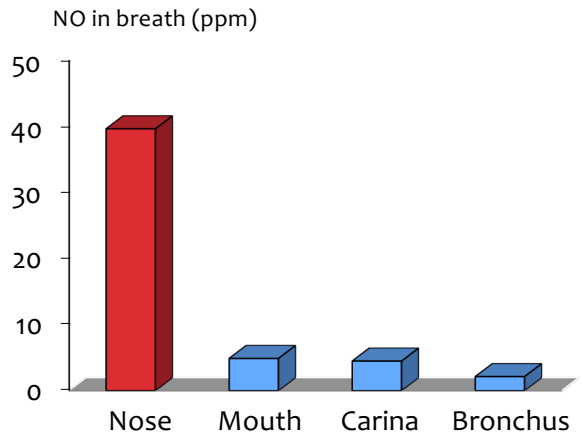
- Diffuse modulation
- NANC
- Retrograde messenger
  (confirms message receipt to the sender)
- Long-term potentiation
  (presynaptic cell programmed to next send a stronger signal - underlies memory)
- Learning, memory, sleep, pain, depression

NO in breath

NOS most abundant in nose and paranasal cavities

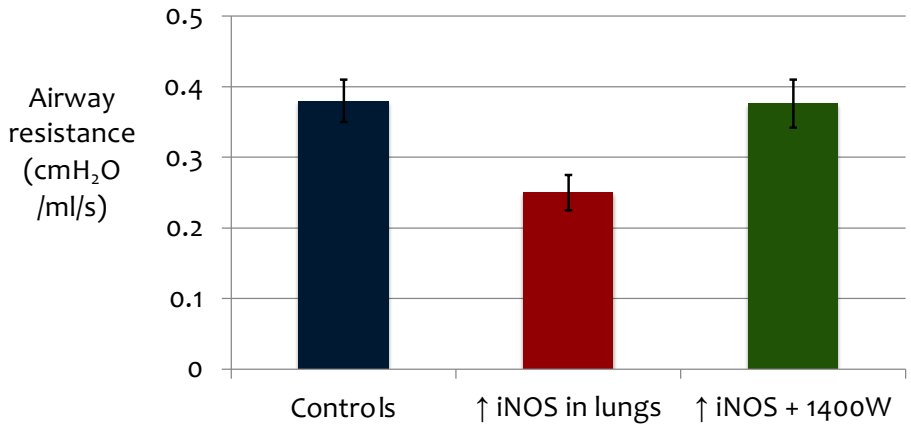
- Desinfection ?
- Bronchodilation
- Regulation of pulmonary vessels?

- Altered in some diseases (e.g. asthma)



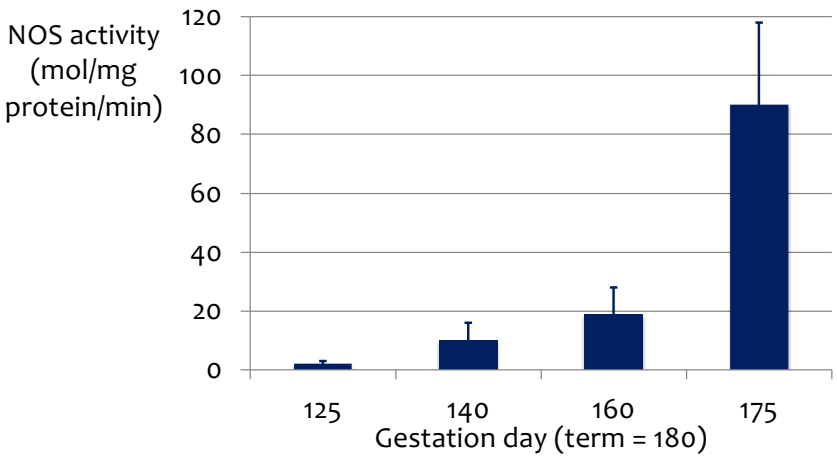
Dillon et al, Chest 1996

NO in airways reduces their resistance



Hjoberg et al: J Appl Physiol 2004

NO in airways rises before birth

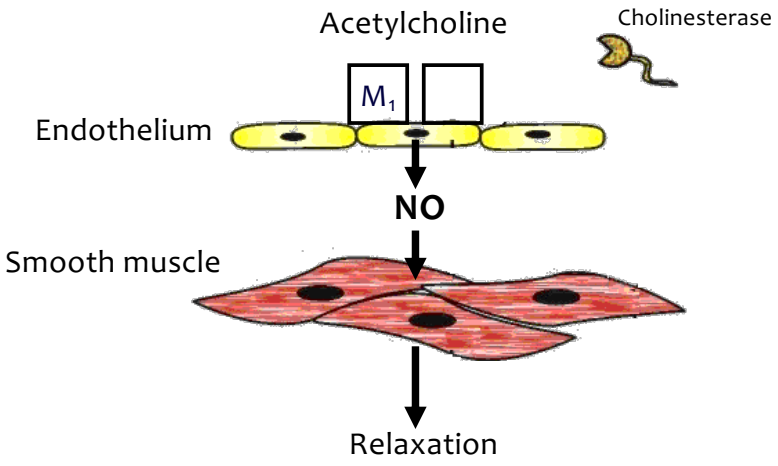


Shaul: Am J Physiol Lung 2002

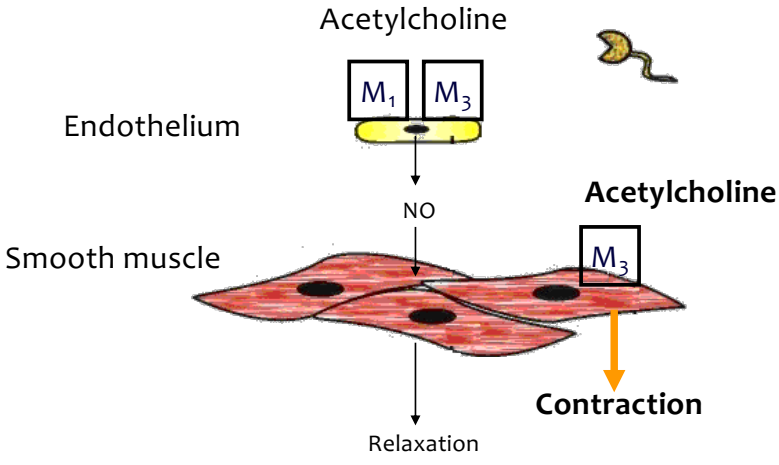
NO is not the only gaseous mediator

- Other gasotransmitters:
  - H_2S
  - CO

Endothelium-dependent vasodilation



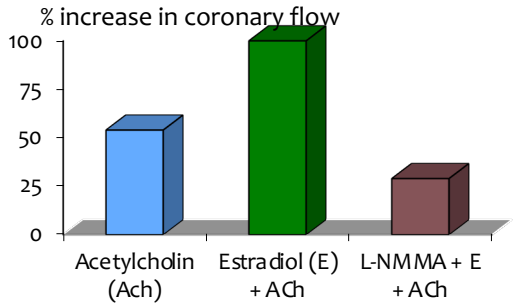
Endothelium-dependent vasodilation



Endothelium-dependent vasodilation

Potentiated by:

- estrogens
  - premenopausal women ↓ risk of cardiovascular diseases
  - after menopause their risk = men
  - ↑ in pregnancy, esp. in uterus (x preeclampsia)



- insulin
  - ↑ glucose delivery to tissues (by ↑ blood flow)

Functions of NO: regulation of blood vessels

Flow-induced vasodilation:

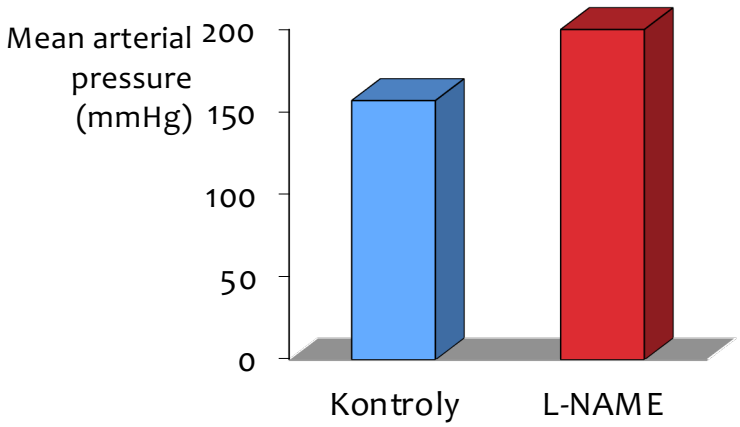
vasodilation in peripheral organs

→ speed of blood flow in more proximal arteries → ↑ shear stress
→ ↑ eNOS activity (& expression)
→ vasodilation in proximal arteries

NO is indispensable in this function

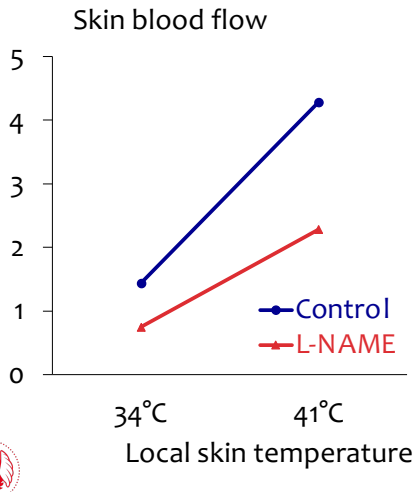
(dysfunction causes hypertension)

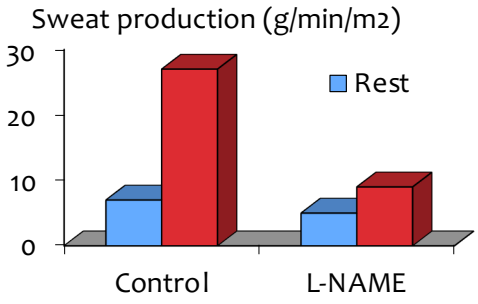
„Tonic “ NO production



Isaacson: J Appl Physiol 1993

NO & thermoregulation

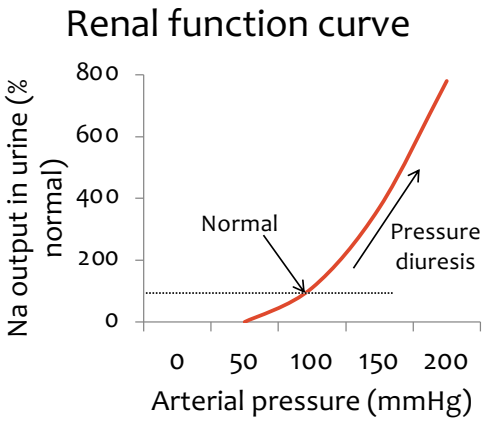




NO in kidneys

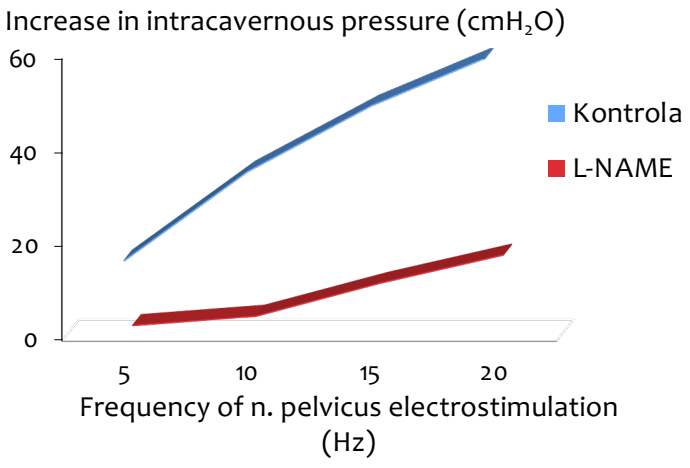
Mediates pressure diuresis:

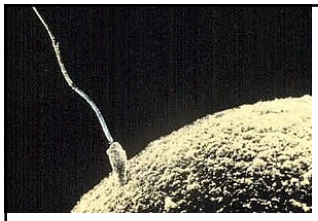
↑ arterial pressure
→ mechanical strain of the endothelium
→ ↑ NO synthesis
→ diffusion to tubules
→ ↓ Na+ reabsorbtion



NO & penile erection

NO from n. pelvici terminals relaxes cavernous smooth muscle





NO and Fertilization

sperm entry into oocyte activates NOS in sperm's acrosome → ↑ NO in oocyte

essential the next steps:

- blocking the entry of additional sperm
- orienting the pronuclei for fusion

NO reduces blood clotting

- inhibition of platelet adhesion, aggregation and secretion
- activated platelets also form NO - feedback inhibition of aggregation

phylogenetically old – crabs 500 million years ago (long before mammals)

NO inhibits apoptosis

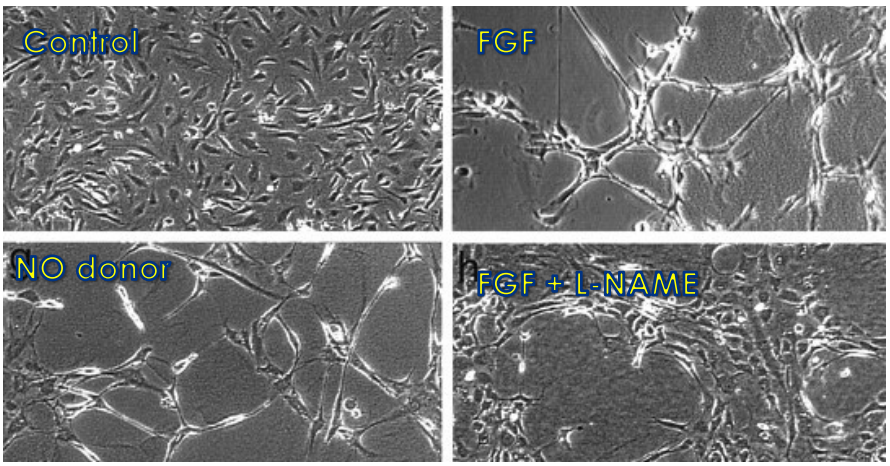
- apoptosis: "physiological" way of cell death
- unlike necrosis, it does not cause inflammation
- NO (→ cGMP → G kinase) inhibits apoptotic phosphorylation signals
- NO directly inhibits caspases
  (specific proteases of apoptosis)

Dual role of NO in ischemia/reperfusion

- small NO amounts protect against ischemic damage
- NO important for preconditioning
- but NO contributes to reperfusion injury (excess NO formed during reperfusion reacts with O_2^- → $ONOO^-$)

NO promotes angiogenesis

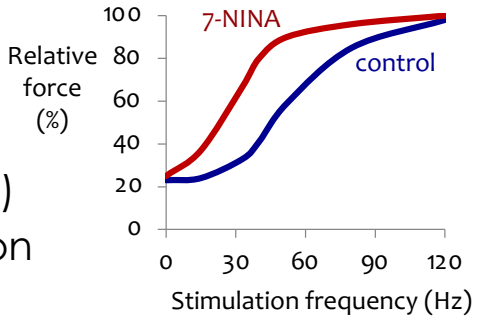
Endothelial cells *in vitro:*



Saeid Babaei et al: Circ. Res. 1998

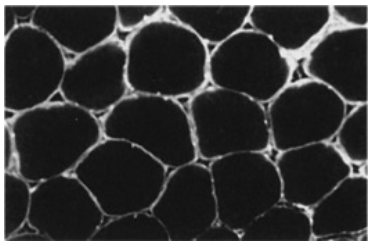
NO in skeletal muscle

- muscle has all NOS isoforms (incl. muscle-specific splicevariant of NOS I)
- NO inhibits contraction (E-C coupling)

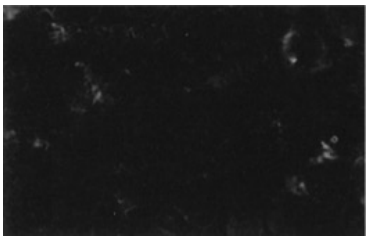


- NO affects autoregulation of blood flow, respiration and glucose homeostasis
- NO modulates myocyte differentiation

Absent nNOS in skeletal muscle in Duchenne muscular dystrophy



health



DMD

Stamler & Meissne: Physiol Rev 2001

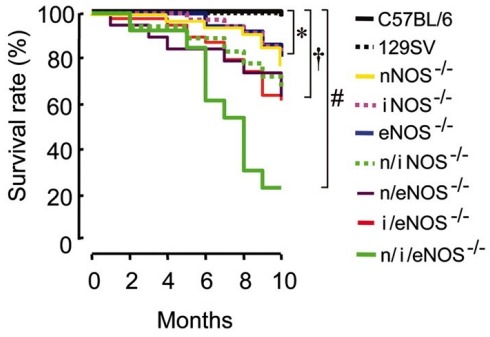
Other NO roles

- NO from the endocardium modulates **cardiac** contractility
- **bone**:
  - lots of NO (e.g., estrogen, exertion):
    - ilnhibition of resorption (by inhibiting osteoclast formation & activity)
  - little NO:
    - potentiation of resorption induced by cytokines
    - probably essential for normal osteoclast function
- involved in **lactation** regulation (?)
- Essential negative regulator of proliferation during development (no differentiation without growth arrest)

NO & longevity

NOS-/- mice:

- premature aging
- ↓ life expectancy



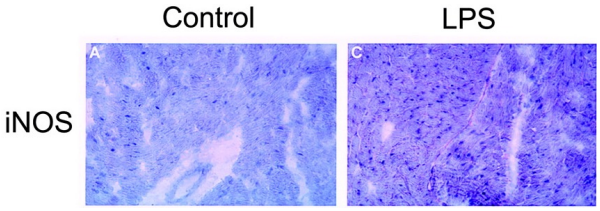
- caloric restriction does not prolong life

NO patofysiology

- septic shock
- hypertenze (?)
- atheroskleróza
- angina pectoris
- záněty, autoimunita
- erektilní dysfunkce
- diabetes mellitus (?)
- mozková mrtvice, roztroušená skleróza, Alzheimer (?), Parkinson (?)

Septic shock

- Infection (endotoxin) induces iNOS expression



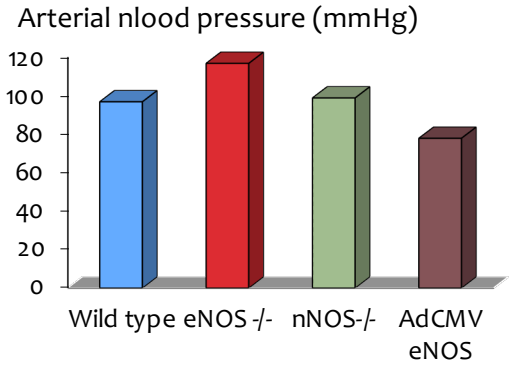
- High NO production:
  - eradication of infection
  - but also vasodilation → massive hypotension
  - iNOS induced in myocardium → ↓ contractility

NO & hypertension

- ↓ eNOS → hypertension

But:

- NO synthesis often not ↓ in hypertension (sometimes ↑)



NO & hypertension: Σ

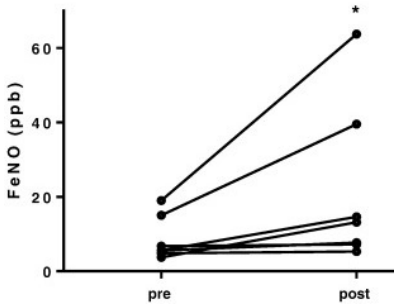
- NO dysfunction is not the primary cause of hypertension
- endothelial damage by high pressure can secondarily reduce NO production
- this then further aggravates hypertension

NO & atherosclerosis

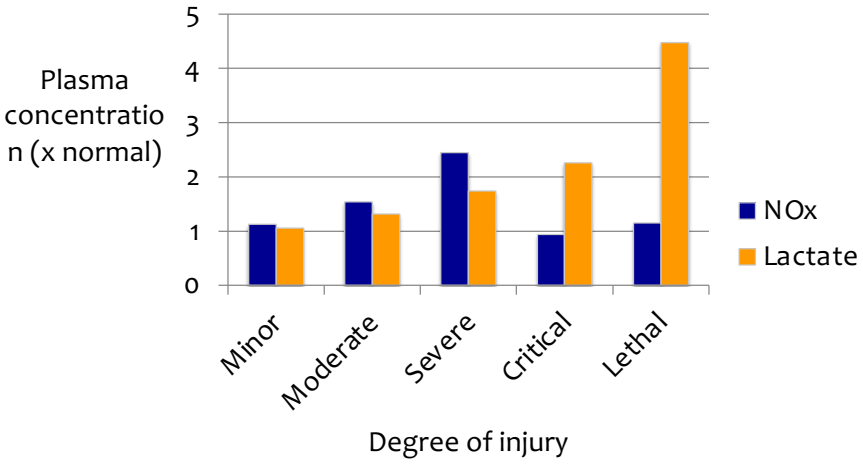
Atherosclerotic plaque
→ endothelial dysfunction
→ ↓ NO production
→ paradoxical vasoconstriction
(e.g. coronary vessels during exercise - angina pectoris)
→ ↓ protection from thrombi generation
→ e.g. MI

NO in diagnostics

- Exhaled NO:
  - airway inflammation
- NO_x in plasma
  - polytrauma



Serum NOx in polytrauma

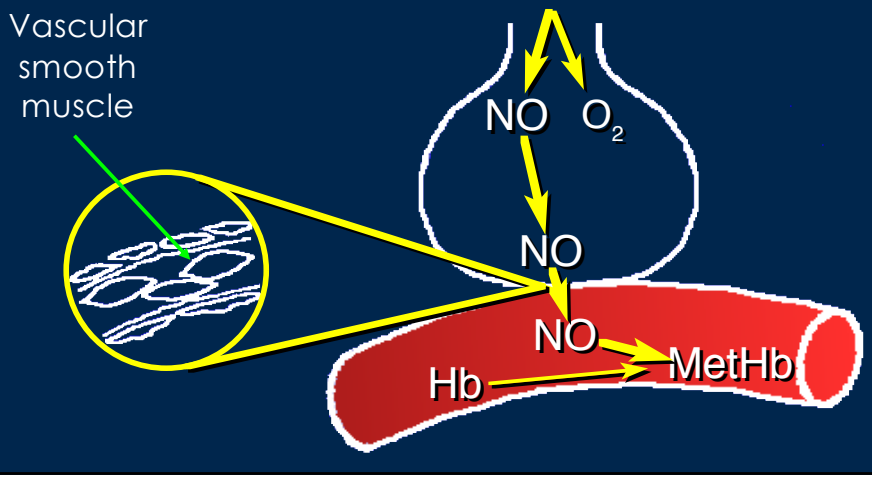


Beitl et al: *Nitric oxide as an indicator for severity of injury in polytrauma, 2016*

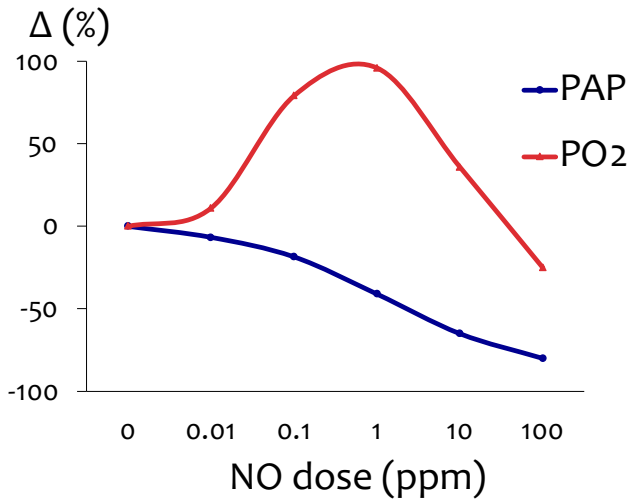
NO in therapy

- Shock - experimental therapy with NOS inhibitors (aminoguanidine)
  (results so far inconclusive)
- Inhalation of NO gas:
  - PPHN
  - ARDS
- Erectile dysfunction - Viagra (PDE V inhibitor)
- NOates

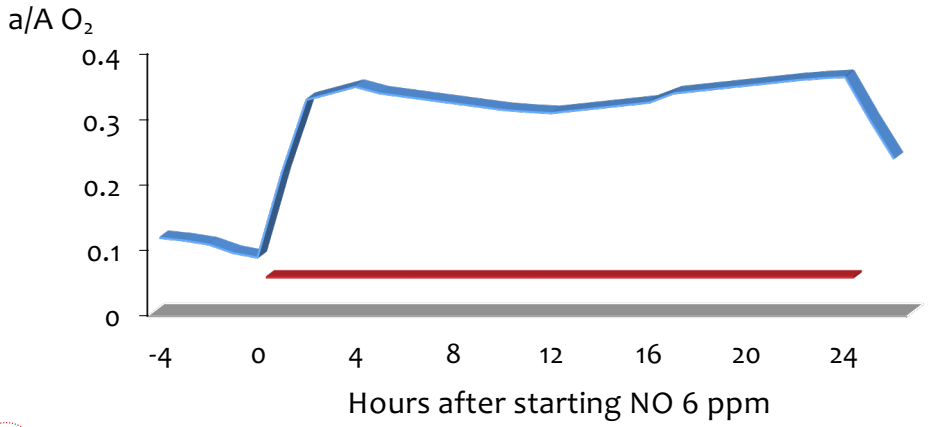
Selectivity of inhaled NO for pulmonary circulation



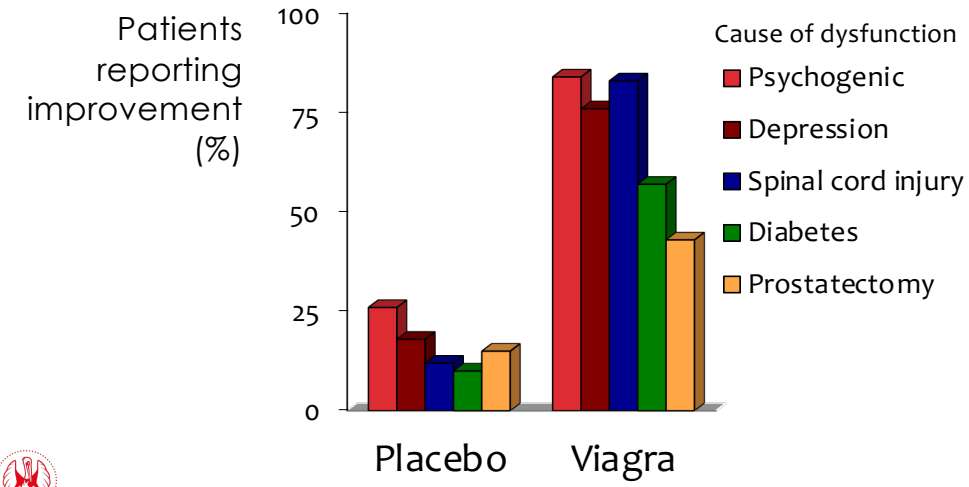
NO inhalation in ARDS



NO inhalation in PPHN



Sildenafil



NOates in pulmonary hypertension therapy