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NOS-1, a Double-Faced Enzyme

by

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Introduction: Nitric Oxide ($NO^{\bullet}$)

- *Science* 1992 “Molecule of the year”.
- A free radical
- Important physiological roles:
  - –Cardiovascular system: controls vasomotor tone.
  - –Nervous system: neural transmitter.
  - –Immune system: inflammatory response.

  Bredt DS. (1999) Endogenous nitric oxide synthesis: biological functions and pathophysiology. *Free Rad Res.* **31:** 577-596
- Synthesized from L-arginine by NO synthase (NOS).

Introduction: The NOS Family

- NOS-1 (nNOS) — *Focus of this presentation*
  - –Primarily in nervous system.
  - –Constitutive expression; Ca^{2+} dependent.
- NOS-2 (iNOS)
  - –Primarily in immune system.
  - –Inducible expression; Ca^{2+} independent.
- NOS-3 (eNOS)
  - –Primarily in cardiovascular system.
  - –Constitutive expression; Ca^{2+} dependent.

Bredt DS. (1999) Endogenous nitric oxide synthesis: biological functions and pathophysiology. *Free Rad Res.* **31:** 577-596.

NOS-catalyzed Generation of $NO^{\bullet}$

L-Arginine

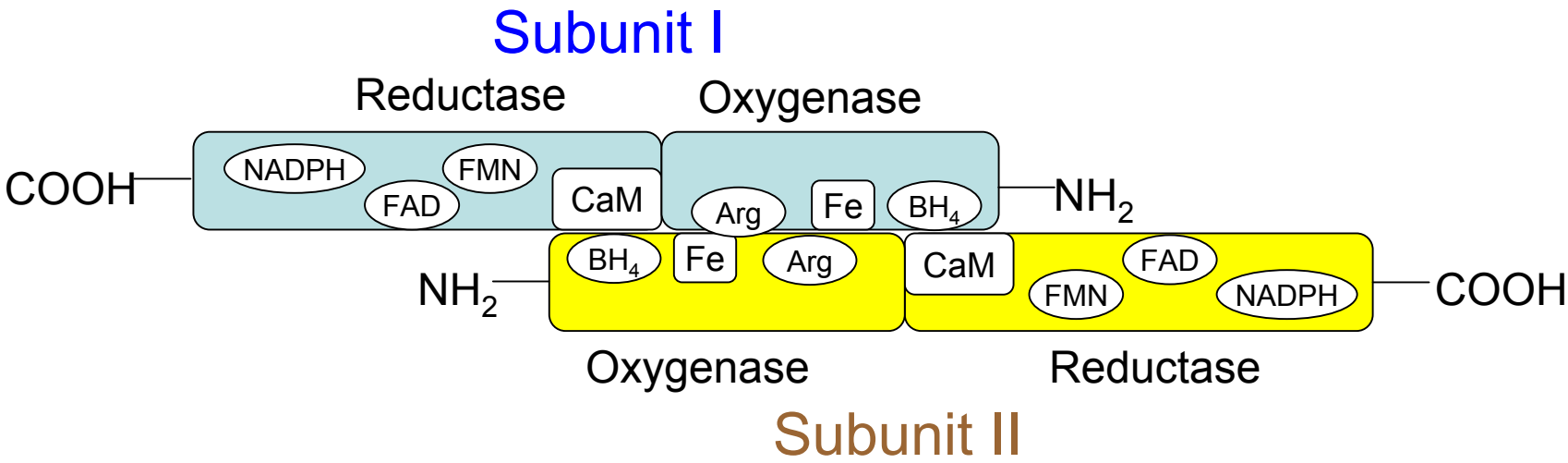
N^{G}-Hydroxy-L-Arginine

L-Citrulline

Nitric Oxide

Adapted from Andrew PJ, Mayer B. (1999) Enzymatic function of nitric oxide synthases. *Cardiovasc Res.* **43:** 521-531.

Common Features of NOS Family



Adapted from Andrew PJ, Mayer B. (1999) Enzymatic function of nitric oxide synthases. *Cardiovasc Res.* **43:** 521-531.

- Catalyze $NO^{\bullet}$ generation from L-arginine:
- N-terminal oxygenase activity and C-terminal reductase activity.
- Act in a dimer form.
- Cofactors: heme, tetrahydrobiopterin (BH_4), NADPH, FAD, FMN, and calmodulin (CaM).

An Overview of NOS-1

- The first identified NOS.

  Bredt DS, Snyder SH. (1990) Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. *Proc Natl Acad Sci USA.* **87:** 682-685.

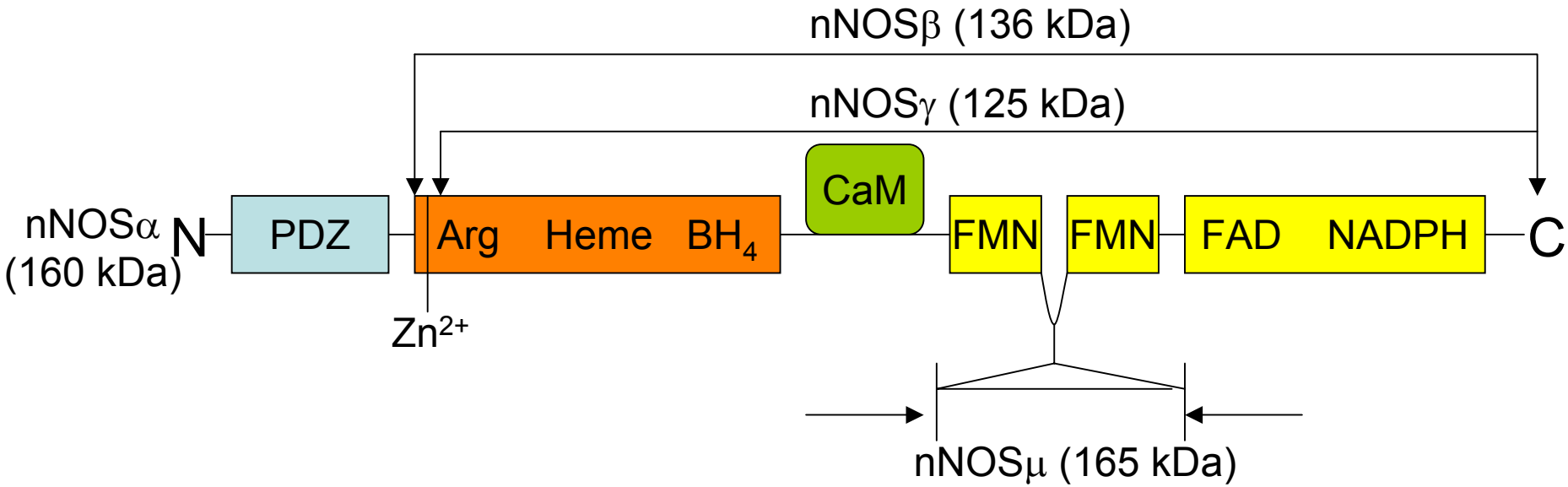
- Human NOS-1 gene contains 29 exons and is located on chromosome 12q24.2.

  Forstermann U, Kleinert H. (1995) Nitric oxide synthase: expression and expressional control of the three isoforms. *Naunyn Schmiedebergs Arch Pharmacol.* **352:** 351-364.

- The physiological roles are controversial:
  - –Important for normal brain functions.
  - –Responsible for brain damage after hypoxia-ischemia.

  Bredt DS. (1999) Endogenous nitric oxide synthesis: biological functions and pathophysiology. *Free Rad Res.* **31:** 577-596.

NOS-1 Biochemistry: Functional Elements and Isoforms



Adapted from Andrew PJ, Mayer B. (1999) Enzymatic function of nitric oxide synthases. *Cardiovasc Res.* **43:** 521-531.

- Has different domains binding to different cofactors, which are important for its function.
- Four isoforms:
  - With PDZ domain: nNOSα, nNOSμ;
  - Without PDZ domain: nNOSβ, nNOSγ.

NOS-1 Biochemistry: The PDZ Domain

- A 90-residue protein-recognition module.
- Mediates binding to proteins with an Asp-X-Val consensus (e.g. glutamate and melatonin receptors).
- Determines the sub-cellular localizations of NOS-1 isoforms:
  - –nNOSα and nNOSμ: membrane bound.
  - –nNOSβ and nNOSγ: cytoplasm.

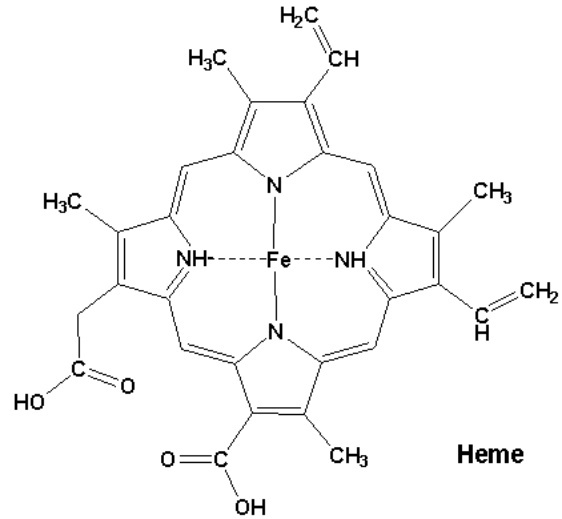
Andrew PJ, Mayer B. (1999) Enzymatic function of nitric oxide synthases. *Cardiovasc Res.* **43:** 521-531.

Human NOS-1 PDZ domain

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

http://www.tsurumi.yokohama-cu.ac.jp/biophys/gallery.html (Mar 16, 2005)

NOS-1 Biochemistry: The Roles of Heme



www.aw-bc.com/ mathews/GH/HEME.GIF(Mar 16, 2005)

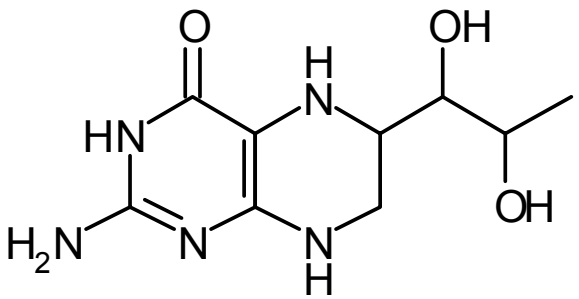
- Heme binds to NOS-1 through a proximal cysteine thiolate ligand.
- The binding of heme is a sole requirement for the formation of active NOS-1 dimers.

  Klatt P *et al*. (1996) Characterization of heme-deficient neuronal nitric oxide synthase reveals a role of heme in subunit dimerization and binding of the amino acid substrate and tetrahydrobiopterin. *J Biol Chem.* **271:** 7336-7342.

- Heme is also essential for the interaction between the oxygenase and reductase domains.

  Andrew PJ, Mayer B. (1999) Enzymatic function of nitric oxide synthases. *Cardiovasc Res.* **43:** 521-531.

NOS-1 Biochemistry: The Roles of BH_4



BH_4

- Stablization of the NOS-1 dimer.

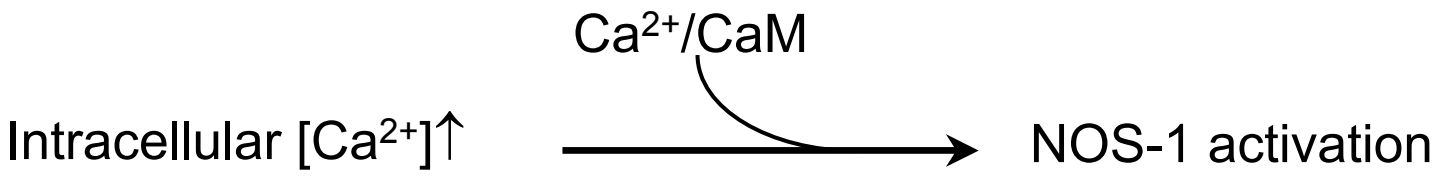
  Klatt P *et al*. (1995) Structural analysis of porcine brain nitric oxide synthase reveals a role for tetrahydrobiopterin and L-arginine in the formation of an SDS-resistant dimer. *EMBO J.* **14:** 3687-3695.

- Possible roles in electron transfer.

  Andrew PJ, Mayer B. (1999) Enzymatic function of nitric oxide synthases. *Cardiovasc Res.* **43:** 521-531.

- Full roles are not completely understood.

NOS-1 Biochemistry: Ca^{2+} Dependence and Calmodulin



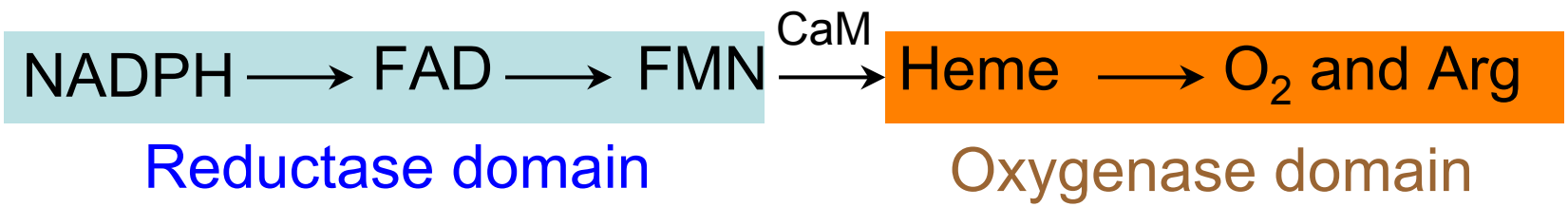
- The Ca^{2+} dependence is due to an autoinhibitory sequence in the FMN-binding region, which prevents CaM binding.

  Salerno JC *et al*. (1997) An autoinhibitory control element defines calcium-regulated isoforms of nitric oxide synthase. *J Biol Chem.* **272:** 29769-29777.

- Only Ca^{2+}-binding CaM activates NOS-1.
- CaM stimulates the electron transfer in the reductase domain, and is critical for the electron transfer to heme.

  Abu-Soud HM, Yoho LL, Stuehr DJ. (1994) Calmodulin controls neuronal nitric oxide synthase by a dual mechanism: activation of intra-and interdomain electron transfer. *J Biol Chem.* **269:** 32047-32050.

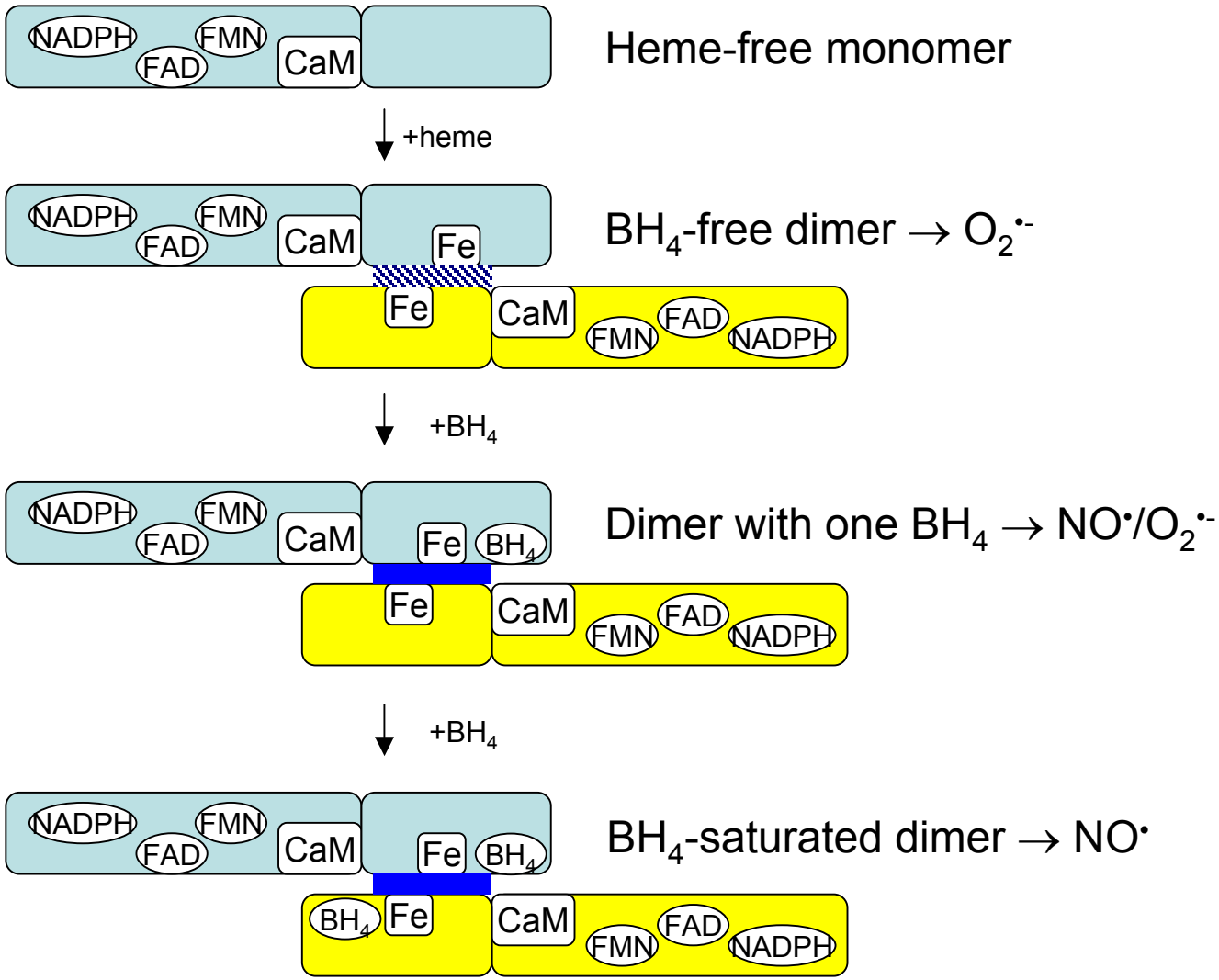
Electron Transfer During $NO^{\bullet}$ Synthesis



- Electron donor: NADPH.
- CaM plays an essential role in the transdomain electron transfer.
- The products of NOS-1-catalyzed reaction vary, depending upon the availability of L-Arg and different stages of enzyme assembly.
- Low concentration or absence of L-arginine, $O_2^{\bullet -}$ and H_2O_2 are produced (the uncoupled reaction).

Andrew PJ, Mayer B. (1999) Enzymatic function of nitric oxide synthases. *Cardiovasc Res.* **43:** 521-531.

Different Assembly Stage, Different Products



Redrawn from: Andrew PJ, Mayer B. (1999) Enzymatic function of nitric oxide synthases. *Cardiovasc Res.* **43:** 521-531.

Is NOS-1 a Peroxynitrite Synthase?

- The *in vitro* purified NOS-1 is in the "dimer with one BH_4" stage. In saturating L-Arg concetrations, it tends to produce $NO^{\bullet}$ and $O_2^{\bullet -}$ at the same time.

  Andrew PJ, Mayer B. (1999) Enzymatic function of nitric oxide synthases. *Cardiovasc Res.* **43:** 521-531.

- This will be dangerous *in vivo*, because:

$$NO^{\bullet} + O_2^{\bullet -} \rightarrow ONOO^{-} \ (k = 1 \times 10^{10})$$

$$ONOO^{-} + CO_2 \rightarrow NO_2^{\bullet} + CO_3^{\bullet -}$$

  Beckman JS, Koppenol WH. (1996) Nitric oxide, superoxide, and peoxynitrite: the good, the bad, and the ugly. *Am J Physiol*. **40:** C1424-C1437.

- The presence of GSH and SOD prevent the formation of peroxynitrite *in vivo*.

  Andrew PJ, Mayer B. (1999) Enzymatic function of nitric oxide synthases. *Cardiovasc Res.* **43:** 521-531.

Measurement of NOS-1 Activity

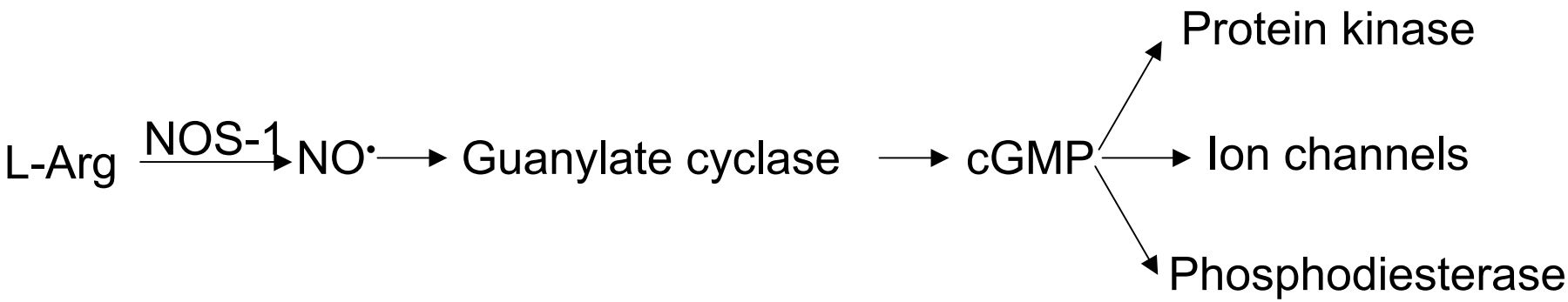
- $NO^{\bullet}$ production: Simply by nitrate level cannot exclude $NO^{\bullet}$ from other sources. To be specific, use L-[$^{15}N_2$]arginine.

  Forte P, Smith LM, Milne E, Benjamin N. (1999) Measurement of nitric oxide synthesis in humans using L-[^{15}N2]arginine. *Methods Enzymol.* **301:** 92-98.

- $O_2^{\bullet -}$ production: EPR.

  Vasquez-Vivar J *et al*. (1999) Electron spin resonance spin-trapping detection of superoxide generated by neuronal nitric oxide synthase. *Methods Enzymol.* **301:** 169-177.

NOS-1 in Physiology: the Useful Aspects



- CNS development: synaptogenesis (this role can be compensated by other NOS isoforms).

  Williams CV, Nordquist D, McLoon SC. (1994) Correlation of nitric oxide synthase expression with changing patterns of axonal projections in the developing visual system. *J Neurosci.* **14:** 1746-1755.

- Learning and memory: regulation of synaptic plasticity.

  Zorumski CF, Izumi Y. (1993) Nitric oxide and hippocampal synaptic plasticity. *Biochem Pharmacol*. **46:** 777-785.

- Cerebral blood flow: regulation of smooth muscle in the blood vessel.

  Faraci FM, Brian Jr JE. (1994) Nitric oxide and the cerebral circulation. *Stroke*. **25:** 692-703.

NOS-1 in Pathology: the Harmful Aspects

- Overdose of $NO^{\bullet}$ leads to toxicity: inhibition of a variety of enzymes.

  Dawson VL *et al*. (1991) Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proc Natl Acad Sci USA.* **88:** 6368-6371.

- Peroxynitrite generation: highly reactive, reacts with CO_2, resulting in more reactive products.
- $NO^{\bullet}$ from NOS-1 is implicated in brain damage following hypoxia-ischemia and during AIDS.

  Dawson TM, Snyder SH. (1994) Gas as biological messengers: nitric oxide and carbon monoxide in the brain. *J Neurosci.* **14:** 5147-5159.

  Dawson VL *et al*. (1993) Human immunodeficiency virus type 1 coat protein neurotoxicity mediated by nitric oxide in primary cortical cultures. *Proc Natl Acad Sci USA.* **90:** 3256-3259.

NOS-1 Knockout Mice

- Knockout of nNOSα, nNOSμ: viable, with enlarged stomachs and complicated behavioral abnormality. However, the effects of the remaining nNOSβ, nNOSγ need to be considered.

  Mungrue IN, Bredt DS, Stewart DJ, Husain M. (2003) From molecules to mammal: what's NOS got to do with it? *Acta Physiol Scand*. **179:** 123-135.

- Complete knockout of NOS-1: viable, defects in reproductive physiology. Its roles in CNS seem to be compensated by other NOS forms.

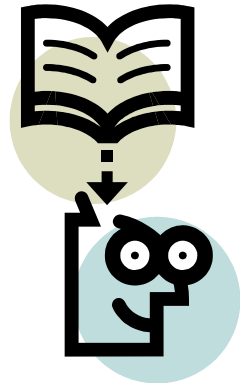
  Gyurko R, Leupen S, Huang PL. (2002) Deletion of exon 6 of the neuronal nitric oxide synthase gene in mice results hypogonadism and infertility. *Endocrinology*. **143:** 2767-2774.

NOS-1 Inhibitors

- Could be useful in treatment of CNS disorders.
- Non-selective (inhibit all three forms of NOS): arginine analogues; thiocitrullines; substituted guanidoamine; thioureas and so on.
- Relatively selective for NOS-1: indazoles; A-84643; ARL-17477, but still all have unwanted side effects on human.

Resink A, Dawnson VL, Dawnson TM. (1996) Nitric oxide synthase inhibitors: future therapy for CNS disorders? *CNS Drugs.* **6 (5):** 351-357.

Summary and Conclusions



- NOS-1 is able to produce both $NO^{\bullet}$ and $O_2^{\bullet -}$, which forms the basis for its roles in both physiologic and pathologic processes.

- Prevention of its harmful effects requires a better understanding of this enzyme and its cofactors.