BioChem 330 - Course Outline

• **Signal Transduction:** short acting signals.
  –**Nitric Oxide** is a fast acting gaseous signaling molecule responsible for a variety of functions.
  –**Nitric Oxide Synthase, NOS** the enzyme which synthesizes NO occurs in three isoforms and has a complex structure
  –**Reaction catalyzed:** reaction of the amino acid arginine with molecular oxygen to form citrulline and NO
What does Nitric Oxide do?

– is implicated in learning, memory
– control of blood pressure
– control of the inflammatory response
– many other biological phenomena
– responsible for more emergency room deaths than any other single molecule
Nitric oxide (NO) is a gas that transmits signals in the organism. Signal transmission by a gas that is produced by one cell, penetrates through membranes and regulates the function of another cell represents an entirely new principle for signaling in biological systems. The discoverers of NO as a signal molecule are awarded this year's Nobel Prize.
Nitric Oxide Synthase, NOS

- occurs in three distinct isoforms:
  - neuronal **NOS, nNOS** in the brain and nerve cells implicated in olfaction, learning
    - chromosome at position 12q24.2
  - epithelial **NOS, eNOS** in the linings of the blood plasma membranes, helps regulate blood pressure
    - chromosome 7 as position 7q35-36
  - inducible **NOS, iNOS** in the inflammatory response, powerfully destructive
    - chromosome 17 at position 17cen-q11.2
Nitric Oxide Synthase, NOS

• Both underproduction and overproduction of NO have been linked to various human pathologies.
  – Impaired NO bioavailability from eNOS and nNOS can lead to hypertension, impotence, or atherosclerosis
  – excess NO production by iNOS can cause inflammation, rheumatoid arthritis, inflammatory bowel disease, immune-type diabetes, stroke, cancer, and death from shock (1/10 intensive care room deaths)
Neuronal Nitric Oxide Synthase, nNOS

- regulator of skeletal muscle contractility, owing to the association of nNOS with the dystrophin-associated glycoprotein complex
- pathology is Duchene muscular dystrophy
- 29 exons spans a region greater than 240 kb
Epithelial Nitric Oxide Synthase, eNOS

Kyoto study shows Glu298Asp mutation correlated with hypertension (Hypertension. 1998; 32)
Nitric Oxide Synthase, the switch is Ca^{++}

- **calcium regulation**
  - Both nNOS and eNOS are constitutive (always in the cell) and calcium modulated, through calmodulin.
  - iNOS, is induced upon trauma, and does not need an influx of calcium for activation. Massive amounts of NO are produced leading to catastrophic cell death (septic and toxic shock).
Reac8on Catalyzed by NOS

- 2O₂ are reduced to 2H₂O (8 electrons gained)
- 1 N on side group of arginine is oxidized to the radical gas NO (5 electrons lost) plus citrulline
- 3 additional electrons provided from 1.5 mobile NADPH but how?
NOS - two distinct domains

- **N-terminal Arg oxidase** is similar in structure to a glove.
- Binds substrates arginine and O₂ and contains:
  - a thiol bound heme group is accessible to solvent; O₂ and Arg bind nearby
  - tetrahydrobiopterin molecule.
  - Structurally important anion, sulfate or phosphate which adjusts 2nd structure
  - Zn(II) ion which resides at the dimer interface.
NOS - two distinct domains

- N-terminal Arg oxidase is
NOS - two distinct domains

- C-terminal collector of electrons
- Binds mobile NADPH, released as NADP+
- Bound cofactor molecules are all bound close to one another and thought to funnel in electrons.
  - NADPH
  - FMN
  - FAD
- Also has p450 site
NOS – domains linked by CaM binding tether

- The electron flow in the ·NO synthase reaction is shown at below.
- CaM binding to nNOS has been shown to regulate catalytic activity by triggering electron flux from FMN to heme, thereby coupling the oxygenase and reductase domains.
- The continual activity of iNOS is explained by its exceptionally high avidity for CaM.
What does NO do in memory?

**Formation of olfactory memories mediated by nitric oxide**


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Sheep learn to recognize the odours of their lambs within two hours of giving birth, and this learning involves synaptic changes within the olfactory bulb. Specifically, mitral cells become increasingly responsive to the learned odour, which stimulates release of both glutamate and GABA (\(\gamma\)-aminobutyric acid) neurotransmitters from the reciprocal synapses between the excitatory mitral cells and inhibitory granule cells. Nitric oxide (NO) has been implicated in synaptic plasticity in other regions of the brain as a result of its modulation of cyclic GMP levels. Here we investigate the possible role of NO in olfactory learning. We find that the neuronal enzyme nitric oxide synthase (nNOS) is expressed in both mitral and granule cells, whereas the guanylyl cyclase subunits that are required for NO stimulation of cGMP formation are expressed only in mitral cells. Immediately after birth, glutamate levels rise, inducing formation of NO and cGMP, which potentiate glutamate release at the mitral-to-granule cell synapses. Inhibition of nNOS or guanylyl cyclase activity prevents both the potentiation of glutamate release and formation of the olfactory memory. The effects of nNOS inhibition can be reversed by infusion of NO into the olfactory bulb. Once memory has formed, however, inhibition of nNOS or guanylyl cyclase activity...
Selective NOS inhibitors are drug targets

- Drug companies filing for patents – basis for Viagra

Male mice without the gene that enables the brain to make a key neurotransmitter attack each other relentlessly, even fatally.
Inhibit iNOS and NOT eNOS and you can prevent septic shock!

Structural characterization of nitric oxide synthase isoforms reveals striking active-site conservation

Thierry O. Fischmann¹, Alan Hruza¹, Xiao Da Niu², James D. Fossetta², Charles A. Lunn², Edward Dolphin², Andrew J. Prongay¹, Paul Reichert¹, Daniel J. Lundell², Satwant K. Narula² and Patricia C. Weber¹

Crystal structures of human endothelial nitric oxide synthase (eNOS) and human inducible NOS (iNOS) catalytic domains were solved in complex with the arginine substrate and an inhibitor S-ethylisothiourea (SEITU), respectively. The small molecules bind in a narrow cleft within the larger active-site cavity containing heme and tetrahydrobiopterin. Both are hydrogen-bonded to a conserved glutamate (eNOS E361, iNOS E377). The active-site residues of iNOS and eNOS are nearly identical. Nevertheless, structural comparisons provide a basis for design of isozyme-selective inhibitors. The high-resolution, refined structures of eNOS (2.4 Å resolution) and iNOS (2.25 Å resolution) reveal an unexpected structural zinc situated at the intermolecular interface and coordinated by four cysteines, two from each monomer.
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