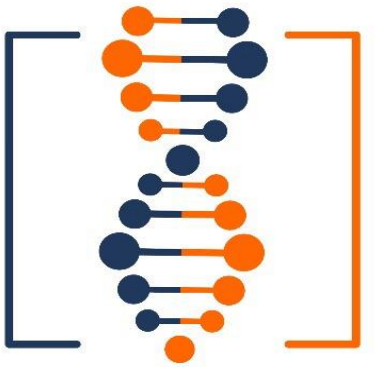


DESTINATION PHARMAGENS

Nitric oxide

Nitric oxide:Biosynthesis

Nitric oxide synthase (NOS) enzymes are central to the control of NO biosynthesis.

There are three known isoforms of NOS:

- **inducible form (iNOS or NOS-II**; expressed in macrophages and Kupffer cells, neutrophils, fibroblasts, vascular smooth muscle and endothelial cells in response to pathological stimuli such as invading microorganisms).
  iNOS produces much greater amounts both because of its high activity and because of its abundance, at least in pathological states associated with cytokine release.
  In contrast to **constitutive NOS isoforms**, the activity of **iNOS** is effectively independent of [Ca2+]i, being fully activated even at the low values of [Ca2+]i present under resting conditions. Induction of **iNOS** is inhibited by glucocorticoids and by several cytokines, including transforming growth factor-β.
- Two so-called **constitutive isoforms**, which are present under physiological conditions in endothelium (**eNOS or NOS-III**) and in neurons (**nNOS or NOS-I**). ***The constitutive enzymes generate small amounts of NO***.
  eNOS is not restricted to endothelium. It is also present in cardiac myocytes, renal mesangial cells, osteoblasts and osteoclasts, airway epithelium and, in small amounts, platelets. Insulin increases **eNOS** activity via tyrosine kinase activation.

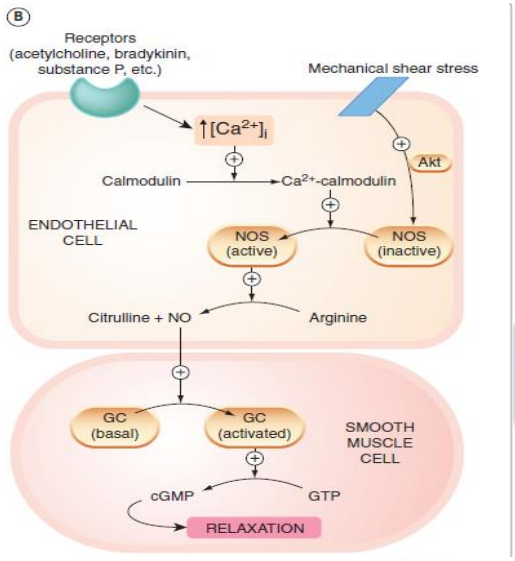
NOS enzymes are functionally 'bimodal', in that they combine oxygenase and reductase activities associated with distinct structural domains. The oxygenase domain contains **haem**, while the reductase domain binds **calcium–calmodulin**.

The activity of **constitutive isoforms of NOS** is controlled by intracellular calcium–calmodulin. Control is exerted in two ways:

1. Many **endothelium-dependent agonists** (e.g. acetylcholine, bradykinin, substance P) increase the cytoplasmic concentration of **calcium ions**, [Ca2+]i; the consequent increase in **calcium–calmodulin** activates **eNOS or nNOS**.

2. **Phosphorylation of specific residues on eNOS** controls its sensitivity to calcium–calmodulin; this can alter NO synthesis in the absence of any change in [Ca2+].

FIG: SEQUENCE OF NO SYNTHESIS



SEQUENCE OF NO SYNTHESIS:

STEPS INVOLVED IN PATHWAY 1

I. Agonist (such as acetylcholine, bradykinin, substance P) mediated increase in intracellular Ca^{2+}
II. Increase in calcicum-calmodulin activated complex
III. Activation of constitutive isoforms eNOS or nNOS of enzymes
IV. L-arginine mediated synthesis of NO

STEPS INVOLVED IN PATHWAY 2

I. Shear stress sensed by endothelial mechanoreceptors and transduced via a serine–threonine protein kinase called Akt.
II. Activation of constitutive isoforms eNOS or nNOS of enzymes
III. L-arginine mediated synthesis of NO.

In **pathological states,** the enzyme can undergo structural change leading to electron transfer between substrates, enzyme co-factors and products becoming 'uncoupled', so that electrons are transferred to molecular oxygen, leading to the synthesis of superoxide anion (O2) rather than NO. This is important, as superoxide anion reacts with NO to form a toxic product (peroxynitrite anion) **L-Arginine** is usually present in excess in endothelial cell cytoplasm, so the rate of production of NO is determined by the activity of the enzyme rather than by substrate availability. Nevertheless, very **high doses of L-arginine can restore endothelial NO biosynthesis in some pathological states.**

PHYSIOLOGICAL ROLE:

1. BIOCHEMICAL AND CELLULAR ASPECTS

- Nitric oxide can activate **guanylyl cyclase** in the same cells that produce it, giving rise to autocrine effects, NO also diffuses from its site of synthesis and activates guanylyl cyclase in neighbouring cells.
- The resulting increase in **cGMP** affects protein kinase G, cyclic nucleotide phosphodiesterases, ion channels and possibly other proteins.
- This inhibits the **[Ca2+]i-induced smooth muscle contraction and platelet aggregation** that occur in response to contractile or pro-aggregatory agonists.
- NO also **hyperpolarises vascular smooth muscle**, as a consequence of **potassium channel activation**.
- NO inhibits **monocyte adhesion and *migration*; adhesion and *aggregation* of platelets**; and smooth muscle and fibroblast proliferation. *These cellular effects probably underlie the anti-atherosclerotic action of NO.*

2. VASCULAR EFFECTS

- The L-arginine/NO pathway is tonically active in resistance vessels. Hence reducing peripheral vascular resistance. As a result systemic blood pressure too.
- Increased NO generation may contribute to the generalised vasodilatation that occurs during pregnancy.

3. NEURONAL EFFECTS

- Nitric oxide is a non-noradrenergic non-cholinergic (NANC) neurotransmitter in many tissues, and is important in the upper airways, gastrointestinal tract and control of penile erection.
- It is implicated in the control of neuronal development and of synaptic plasticity in the CNS.

4. HOST DEFENCE

- Cytotoxic and/or cytostatic effects of NO are implicated in primitive non-specific host defence mechanisms against numerous pathogens, including viruses, bacteria, fungi, protozoa and parasites, and against tumour cells.

Therapeutic use no and no donor

1. **NITRIC OXIDE**

   the main action of inhaled NO is pulmonary vasodilatation, along with vasodilation in ventilated alveoli.

   Use of NO in respiratory distress syndrome (RDS)

- Inspired NO acts preferentially on ventilated alveoli, and could therefore be therapeutically useful in (RDS).
- RDS characterised by intrapulmonary 'shunting' (i.e. pulmonary arterial blood entering the pulmonary vein without passing through capillaries in contact with ventilated alveoli), resulting in arterial hypoxaemia, and by acute pulmonary arterial hypertension.
- Inhaled NO dilates blood vessels in ventilated alveoli (which are exposed to the inspired gas) and thus reduces shunting.
- Ethyl nitrite gas has been investigated in newborns (who are at much increased risk of respiratory distress syndrome because of their immature lungs) as a potentially less toxic alternative.

2. **NITRIC OXIDE DONORS/PRECURSORS**

- **Nitrovasodilators** have been used therapeutically for over a century. The common mode of action of these drugs is as a source of NO.
- There is interest in the potential for selectivity of nitrovasodilators; for instance, **glyceryl trinitrate** is more potent on vascular smooth muscle than on platelets, whereas **S-nitrosoglutathione (SNOG)** selectively inhibits platelet function.
- It was shown recently that dietary nitrate (contained in beetroot juice) acutely lowers arterial blood pressure in parallel with a rise in plasma nitrite concentration and improved endothelial and platelet function.