Nitric Oxide: The Wonder Molecule

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Kushal Chakraborty is a doctoral student at Department of Life Sciences and Biology at Jadavpur University. Presently he is working on the stimulatory effects of various kinds of NSAIDs on different kinds of cells and isolation of that protein from those cells.

Nitric oxide, a newly discovered biological messenger molecule that is produced from different kinds of cells has diversified and has significant effects on various pathological and physiological events ranging from the prevention of cancer and diabetes mellitus to coronary artery disease (heart attack) and hypertension.

Nitric oxide (NO), an inorganic molecule formed by vascular endothelial cells is now thought to be a messenger molecule that is believed to play a crucial role in various biological processes of both physiological and pathological importance.

Nitric oxide is a simple heterodiatomic molecule with broad and diverse functions in human biology that have been recognized only recently. NO is chemically unstable, with a half-life of 3-5 secs in aqueous solution under physiological conditions of concentration, temperature, pH, and O_2 tension. In 1980, Furchgott and Zawadzki reported that a product of the endothelial cell causes vasorelaxation, which is now known as endothelium derived relaxing factor (EDRF) [1]. They showed that the endothelium was obligatory for acetylcholine-elicited relaxation of the isolated rabbit aortic preparation. Based on the pharmacological stimulation of EDRF and NO (generated from acidified NO_2), Furchgott suggested that EDRF may be NO. Comparison of EDRF and NO on vascular strips and on platelets showed that the two compounds were indistinguishable and had identical chemical stability under artificial conditions as determined by the rate of decay during their transit in propylene tubes. Both of them inhibited platelet aggregation and adhesion and promoted segregation of aggregated platelets [2]. In addition, their biological half-lives, as inhibitors of platelet aggregation were similar. EDRF and NO act on platelets and smooth muscle cells by stimulating the soluble guanylate cyclase and

Keywords
Nitric oxide, *l*-arginine, nitric oxide synthase.

Suggested Reading

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elevating the level of cyclic GMP. However, many investigators have debated whether or not NO and EDRF are one and the same. Some reported that EDRF only relaxes the vascular smooth muscle, whereas NO relaxes the vascular and tracheal and the smooth muscles of *Taenia coli*. EDRF has been reported to be stabilized by acidification, a condition that does not stabilize NO.

NO acts near its point of release, entering the target cell and activating the cytosolic enzyme guanyl cyclase, which catalyses the formation of the second messenger cGMP [3].

NO is synthesized by a family of enzymes known as the nitric oxide synthases. Its synthesis is stimulated by interaction of nitric oxide synthase with Ca^{2+}- calmoudulin [4]. Three distinct isoforms have been identified, of which two are named after the cell types from which they were first cloned: neuronal NOS (nNOS, NOS1 gene product); inducible NOS (iNOS, NOS2 gene product), present in monocytes, macrophages, smooth muscle cells, microvascular endothelial cells, fibroblasts, cardiomyocytes, hepatocytes, and megacryocytes; and endothelial NOS (eNOS, NOS3 gene product) [3]. In 2000, Sinha and others have reported the existence of a insulin activated membrane-bound enzyme, a constitutive form of nitric oxide synthase (IANOS), which occurs in a wide variety of cells including human RBC or their membrane fraction but not cytosolic fractions and heart including liver, kidney and muscle intestine in mice [5].

NO is sufficiently non-polar to cross plasma membranes without a carrier. Previously known as a component of smog, this simple gaseous substance diffuses readily through membranes, although its high reactivity limits its diffusion to about 1 mm radius from the site of synthesis. NO is synthesized from arginine in an NADPH-dependent reaction catalysed by nitric oxide synthase, a dimeric enzyme structurally related to NADPH, cytochrome p-450 reductase. The reaction is a five-electron oxidation. Each subunit of the enzyme contains one bound

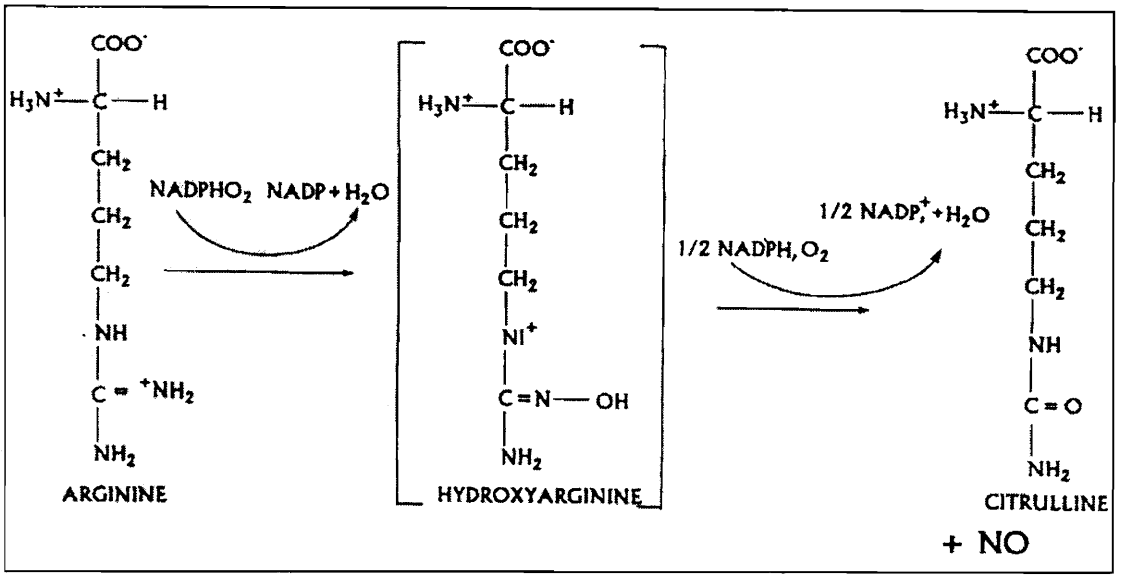

Figure 1. Biosynthesis of nitric oxide. Both steps are catalysed by nitric oxide synthase. The nitrogen of the NO is derived from the guanidine group of arginine.

molecule of each of the four different cofactors FMN, FAD, Tetrahydrobiopterin and Fe^{3+} heme.

It is thought that hemoglobin (Hb) is involved in limiting the NO's point of action; in the process the functional properties of Hb are not altered to any significant degree.

Chemistry of Nitric Oxide

NO can be formed nonenzymatically in numerous laboratory reactions that would be most unlikely to occur in biological tissues. The two most common reactions are

$$Fe^{2+} + NO_2 + 2H^+ \rightarrow Fe^{3+} + NO + H_2O$$

and

$$2NO_2 + 2I^- + 4H^+ \rightarrow 2NO + I_2 + H_2O.$$

FAD = Flavin adenine dinucleotide
FMN = Flavin mononucleotide

Nitric oxide can undergo numerous reactions, as it can act both as Lewis base and a Lewis acid [6]. It also can act both as an oxidizing and a reducing agent.

Some of the more important chemical reactions involving NO are discussed below.

a. Reaction with oxygen to yield NO_2 gas or NO_2^- in solution.

Box 1.

Molecule oxygen binds as superoxide anion to the heme iron atom of hemoglobin [9], and this species reacts rapidly with NO to yield the peroxonitrite anion, which rapidly isomerises to NO_3^-. This principle is used for the spectrophotometric assay of NO [8].

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

b. Reaction with superoxide anion (O_2^-) to yield the unstable intermediate anion $(OONO)^-$, which rearranges to form NO_3

$$NO + O_2 \rightarrow {}^-OONO \rightarrow NO_3$$

This reaction represents one of the few examples of a radical-radical coupling of O_2 with another odd-electron species to generate a diamagnetic product.

c. Reaction with ozone (O_3) to yield an activated or high-energy state NO_2. The activated NO_2 can be readily detected by chemiluminescence, which is used to assay NO (see *Box* 1) [7].

d. Reactivity with oxyhemoglobin to yield methemoglobin and inorganic nitrate.

$$HbO_2 + NO \rightarrow \text{met Hb} + NO_3$$

The components of the above reaction are

$$Hb^{3+}O_2 + NO \rightarrow \text{met Hb} + {}^-OONO$$

and

$${}^-OONO \rightarrow NO_3$$

The earliest evidence that elicits important actions was the finding that NO and nitroso compounds could activate cytoplasmic or cytosolic guanylate cyclase and stimulate cGMP formation in mammalian tissues [9,10]. Various nitrogen-containing substances were found to produce the same effects, and NO was suggested to be the common factor responsible. In experiments designed to elucidate the action of cGMP on human platelet function, NO elicited potent and marked inhibitory effects on platelet aggregation. Studies revealed that NO not only inhibits platelet aggregation but also inhibits platelet adhesion to vascular endothelial surfaces.

Biological Importance of NO

Both the excess and deficiency of NO are believed to be involved in several pathophysiological states. NO is a critical determinant of basal vascular tone, and a deficiency of NO is associated with hypertension. Deficiency of NO manifests in common

disorders that promote atherosclerosis such as hypertension, hyperlipidemia and diabetes. A deficiency of NO producing neurons in the gastrointestinal tract is believed to be responsible for certain abnormalities in gastrointestinal motility such as Hirschsprung's disease, achalasia, and chronic intestinal pseudo obstruction. NO produced by hepatocyte iNOS plays a role in protection of these cells against a variety of hepatic toxins, including ethanol and acetamenophen.

Therapeutic manipulation of NO level has been used to provide exogenous NO to dysfunctional coronary arteries. NO as gas may be useful at concentration of 10 to 40 ppm for the treatment of persistent pulmonary hypertension of the new born. In primary pulmonary hypertension nitric oxide is generally administered via inhalation in 5 to 10 ppm levels and increased every few minutes until no further effectiveness is obtained.

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