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Review

The profound influence of nitric oxide on intercellular communication and health

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ABSTRACT

Nitric oxide (NO) is a short-lived and highly reactive free radical in nitrogen monoxide structure, formed by the combination of nitrogen (N) and oxygen (O) gases in the atmosphere, and is a molecule that can pass through membranes by diffusion. Due to its lipid solubility feature, it does not require a special carrier, and therefore, when it is released from the vascular endothelium, it can easily pass through the cell membrane and relax in the vascular smooth muscle cells. Nitric oxide is formed by the synthesis of L-arginine, an essential amino acid, as a substrate, by NO synthase enzymes. Endothelial-derived relaxing factor (EDRF) causes relaxation of vascular smooth muscle as it activates soluble guanylyl cyclase and then increases cyclic guanosine monophosphate (cGMP) in vascular smooth muscle. It is structurally in the form of a compound containing NO or nitrogen oxide. Additionally, NO is formed from L-arginine by an enzyme bound to calcium-calmodulin and nicotinamide adenine dinucleotide phosphate. Endothelial-derived relaxing factor functions as a vasodilator that serves as an inhibitor of platelet aggregation and adhesion. Furthermore, EDRF serves as a secondary messenger for guanylyl cyclase activation and cGMP production. The role of EDRF as a secondary messenger extends to diverse cellular contexts, including cardiovascular tissue, respiratory and renal epithelium, macrophages, cerebellar neurons, and adrenocytes. This review comprehensively addresses the structure, biosynthesis, and impact of NO, renowned for its significant influence on intercellular communication and health.

Keywords: Nitric oxide, nitric oxide isoforms, nitric oxide physiopathology, nitric oxide synthase.

Furchgott and Zavadski[1] in 1980 determined that there is a substance that is synthesized in vascular endothelial cells and relaxes vascular smooth muscle with acetylcholine stimulus, and they named this substance as endothelial-derived relaxing factor (EDRF). Palmer et al.[2] reported in 1987 that a gas called nitric oxide (NO) is responsible for the known biological effects of EDRF. Nitric oxide is a colorless gas that exhibits solubility in water. In oxygen (O_2)-free environments, NO demonstrates notable stability; however, upon exposure to air, it promptly undergoes a reaction with O_2, transforming into nitrogen dioxide (NO_2). This resultant toxic gas has been associated with inducing tissue damage.[3] Nitric oxide, which has important functions in biological events and also has a short half-life (2-30 seconds), is also defined as a free radical because of the unshared electron it carries in its final orbit. However, unlike other free radicals that induce cellular damage across all concentrations, NO contributes to crucial physiological functions of the cell at low concentrations. Nonetheless, excessive and uncontrolled secretion of NO can lead to significant cellular damage.[4]

Recent studies on NO have shown that the functions of this gas are not only limited to its relaxing effect on vascular smooth muscle, but also act as an intracellular messenger molecule in endothelial and nerve cells, and is an effective molecule in phagocytosis in activated immune cells.[5,6]

BIOSYNTHESIS OF NITRIC OXIDE

At the N-terminal portion of a cysteine-thiol-linked heme protein within the oxygenase region

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of nitric oxide synthase (NOS), the reaction involves nicotinamide adenine dinucleotide (NAD). Electron donor nicotinamide adenine dinucleotide phosphate (NADPH) donates two electrons to enzyme-bound flavin adenine dinucleotide (FAD) to reduce flavin mononucleotide (FMN). Thus, the reduced FMN also reduces the Fe^{2+} in heme to Fe^{3+}.[7] After this step, oxygen can be attached to the oxidase enzymes for the oxygenation of L-arginine. Oxidized L-arginine forms N-hydroxyl L-arginine. In the second step of the reaction, N-hydroxyl L-arginine is oxidized once again, resulting in the formation of one mole of NO and one mole of citrulline.[8] The synthesis of NO from the amino acid L-arginine by NOS, a homolog of cytochrome P-450 reductase, involves the utilization of cofactors such as NADPH, FAD, and FMN in conjunction with molecular oxygen. Essential components such as tetrahydrobiopterin are requisite for this enzymatic process.[9,10]

STRUCTURE AND PROPERTIES OF NITRIC OXIDE SYNTHASE

It is known that NO is synthesized from L-arginine with the help of NOS. The NOS enzyme also contains protoporphyrin IX, which contains Fe, such as cytochrome P-450.[11] The physicochemical and kinetic attributes of NOS were systematically investigated across various organs and categorized into two distinct groups based on these characteristics: NO is synthesized from L-arginine and subsequently oxidized by activated NOS. Three distinct isoforms of NOS are known, including neuronal NOS (nNOS) and endothelial NOS (eNOS), both constitutive NOS (cNOS), and inducible NOS (iNOS).[12] The primary physiological role of NO lies in the regulation of vascular tone. Under normal physiological conditions, NO, efficiently cleared by oxyhemoglobin, does not inflict tissue damage. However, iNOS is induced in response to endotoxins and inflammatory cytokines that proliferate in the environment during infection or inflammation. Induced iNOS releases substantial quantities of NO. Elevated NO levels contribute to tissue damage by augmenting the generation of free radicals, thereby detrimentally influencing the progression of the disease.[13,14]

CONSTITUTIVE NITRIC OXIDE SYNTHASE

This isoenzyme of NO is particularly found in vascular endothelial cells, adrenal cortex medulla cells, uterus and intestinal interstitium, urogenital tissues, central and peripheral nervous system cells, and platelets.[1] Any interaction that increases intracellular ionized calcium, activates cNOS, which is not always active and is constantly present in these tissues, by combining calcium with calmodulin, and NO is synthesized.[1,15] However, when compounds that increase the amount of intracellular calcium such as acetylcholine, histamine, bradykinin, serotonin, glutamate, insulin, and substance P are not stimulated, they decrease the amount of intracellular calcium, cNOS is not activated, which stops NO synthesis.[16,17] Therefore, cNOS is responsible for low NO synthesis in normal biological systems. There are two isoforms of structural cNOS known as nNOS and eNOS.[1,2] Calcium calmodulin-dependent NOS is activated by binding to receptors in endothelial cells through physiological stimuli such as NO, a paramagnetic free radical, acetylcholine, bradykinin, glutamate adenosine diphosphate (ADP). Under the catalysis of this enzyme, L-citrulline and NO are synthesized as a result of the reaction of L arginine with oxygen. In this reaction, molecular oxygen uses NADPH as a cofactor.[18]

Nitric oxide, which is formed as a result of this reaction, diffuses into the vascular smooth muscle cells and activates the enzyme by binding to the heme group of the guanylyl cyclase enzyme. The activated guanylyl cyclase enzyme converts guanosine triphosphate to cyclic guanosine monophosphate (cGMP). The cGMP allows calcium ions (Ca^{++}) to enter the storage areas in their cells. Decreased Ca^{++} in the cell stops muscle contraction by allowing actin and myosin to slide over each other. Thus, smooth muscle cells in the vascular endothelium relax and blood vessels expand.[19,20] Three genetically distinct isoforms of NOS have been identified: the constitutive eNOS, produced in low amounts and responsible for the regulation of vascular tone; the constitutive nNOS, also produced in low amounts, governing synaptic formation and neurotransmission; and the iNOS, produced in high amounts, playing a role in immune-inflammatory events and influencing

cell-mediated immune responses. Unlike the nNOS and eNOS isoenzymes, which rely on the Ca^{2+}-calmodulin complex for NO production, iNOS operates independently.[21]

INDUCIBLE NOS

It is specially released from macrophages (monocytes, neutrophils, hepatocytes, and others…) and vascular endothelial cells. Activation of these cells by cytokines such as tumor necrosis factor (TNF), and interleukin (IL)-1, interferon leads to the induction of NOS and the synthesis of NO. In particular, macrophages stimulated by bacterial lipopolysaccharides, interferon alpha, and highly concentrated lipopolysaccharides produce a large amount of NO, causing a cytotoxic or cytostatic effect on foreign cells (such as bacteria, parasites, and tumor cells…). Excessively synthesized NO for a long time causes damage to macrophages and other tissues. The iNOS enzyme is induced by L-arginine analogs and glucocorticoids. This enzyme has been named inducible iNOS or calcium-independent-NOS due to this known feature.[22]

ENDOTEL NOS

This isoenzyme is always found in an inactive form in tissues such as vascular endothelium, urinary tract tissues, and peripheral and central nervous system. When the intracellular ionized Ca^{++} concentration increases, Ca^{++} binds to calmodulin and activates the NOS enzyme, resulting in L-arginine and NO synthesis. However, as soon as the intracellular ionized calcium concentration begins to decrease, the enzyme switches to an inactive form and NO synthesis stops. The enzyme is characterized as calcium-dependent NOS, also referred to as eNOS or cNOS, owing to its activation by the calcium-calmodulin complex.[23]

NEURONAL NOS

Nitric oxide synthesis occurs within neurons located in the cerebellum and forebrain, as well as at the terminations of certain autonomic nerves. Designated as nNOS, this particular isoform of the NOS enzyme is so named due to its localization within neurons.[24] This enzyme is structurally dependent on calcium calmodulin-like eNOS found in the endothelium.[23] Nitric oxide is a neurotransmitter that performs many functions in the central nervous system, such as impulse transmission, balance, memory, and olfaction. However, in the peripheral nervous system, NO contributes to the regulation of the respiratory, urinary, and digestive systems by vasodilation by affecting the noradrenergic and noncholinergic nerves.[25]

PATHOLOGICAL RELEASE OF NITRIC OXIDE

Inducible NOS found in vascular endothelium and smooth muscle can be induced by some cytokines or by endotoxic lipopolysaccharides that increase cytokine release. This induction occurs simultaneously in the vascular endothelium and smooth muscle, resulting in vasodilation. This phenomenon is expressed as a resistance to vase contractures. Hypotension due to increased NO synthesis seen in endotoxic and septic shocks of animals can be given as an example. Moreover, the occurrence of hypotension in cancer patients undergoing cytokine therapy is attributed to the elevated levels of NO induced by this treatment.[26]

However, in these cases, a nitrovasodilator should be given to regulate the hypertension that may occur. This treatment will also prevent platelet aggregation and adhesion that would occur when NO synthesis is blocked.[26,27]

EFFECTS OF NITRITE AND NITRATE COMPOUNDS ON ORGANISMS

Nitrites and nitrates are known as precursors of N-nitroso compounds. It was first determined in 1956 that dimethylnitrosamine causes liver cancer in rats. So far, compounds belonging to the N-nitrosamine class have been identified in over 100 instances as causative agents of cancer in rats.[28]

Nitrites have a toxic effect on oxyhemoglobin by converting it to methemoglobin. Clinical symptoms of anoxia occur when 20-40% of hemoglobin is converted to methemoglobin.[29]

Likewise, studies conducted to investigate whether there is any relationship between nitrite and cancer have stated that high-dose nitrite may cause cancer formation.

NITRIC OXIDE IN PULMONARY AND CARDIOVASCULAR SYSTEM

The main task of the vascular system is to prevent the adhesion and aggregation of platelets and other blood cells and to dilate the blood vessels enough to provide sufficient flow. One of the substances synthesized to achieve this is NO.[30] Nitric oxide is a vasodilator released from healthy endothelial cells. Systemic use of NO synthesis inhibitors increases blood pressure in small arterioles.[29] Nitric oxide not only ensures the regulation of systemic circulation but also assists in the modulation of local circulation in organs such as the heart, liver, and brain. Normal vascular resistance is regulated by endothelium-derived vasodilators [NO, prostacyclin (PGI2)] and vasoconstrictors (endothelin). Under normal conditions, this balance is in the direction of vasodilation.[31] Nitric oxide and PGI2 collaboratively regulate vasodilation and the prevention of platelet aggregation. However, their mechanisms differ; PGI2 induces vasodilation through the second messenger cyclic adenosine monophosphate, while NO operates via the second messenger cGMP.[32]

Nitric oxide, the most important endothelial-derived relaxant factor, is a lipophilic gas secreted from endothelial cells in response to various chemical and physical stimuli. Excessive disruption in NO synthesis causes vasoconstriction, increasing the risk of chronic hypertension and atherosclerosis. In this case, vascular endothelial cell damage due to hypertension may worsen. If left untreated, it can cause vascular damage to sensitive tissues such as the heart, kidney, and brain.[4,33]

Since arterial endothelial tissue synthesizes more NO than vein endothelial tissue, relaxation in arteries is greater than in veins. It is known that disruptions in NO synthesis cause hypertension.[33] As blood flows through arteries and arterioles, a viscous friction of blood against the vessel wall results in a phenomenon called frictional stress in endothelial cells. The resulting stress exerts pressure on the endothelial cells in the direction of flow, significantly increasing NO release. Nitric oxide also causes dilatation by relaxing the arterial wall. The NO formed passes into the smooth muscle cells by simple diffusion and stimulates cGMP as a second messenger, causing the arteries to expand, thus playing a role in the regulation of blood pressure.[34] Nitric oxide synthesis and release in endothelial cells are also stimulated by some vasoconstrictors such as angiotensin II.[33]

Endothelial cells are constantly exposed to injury. Fats, immune compounds, microorganisms, and toxins are the main causes of deterioration of blood vessel integrity and homeostasis of blood composition. Due to endothelial injuries, a reduction in the release of protective molecules like NO leads to the accumulation of adhesive components such as platelets, monocytes, neutrophils, and cholesterol in the intima layer of the vessel. In the absence of NO, smooth muscle cell activation increases, and as a result, cell proliferation leads to atherosclerosis.[35]

Conflicting results have been obtained in studies conducted in humans regarding the effects of L-arginine on vascular tone and blood pressure.[4,33] Systemic infusion of L-arginine has been shown to cause a decrease in blood pressure and an increase in plasma cGMP levels; however, this effect was not observed in other studies.[36,37] Similarly, L-arginine applied to the forearm arterial walls of healthy individuals demonstrated an increased response to acetylcholine infusion. However, this finding could not be detected in all of the other studies.[37] The kidneys play an important role in the pathogenesis of hypertension. Therefore, it is important to evaluate the relationship of NO with kidney functions and kidney diseases. The kidneys are very sensitive to NO. Even the administration of very low doses of L-arginine analog that does not cause a change in blood pressure can cause a decrease in renal blood flow, natriuresis, and diuresis. Therefore, NO changes that do not affect endothelial-induced relaxation are likely to contribute to hypertension by causing changes in fluid-electrolyte levels.[38]

Increased blood pressure or vasodilation induced by acetylcholine; is impaired in patients with atherosclerosis, smokers, and children with familial high cholesterol.[39] It has been observed that administration of L-arginine to these patients improves vascular dysfunction. At the same time, it has been observed that L-arginine infusion in healthy people with hypertension reduces systolic and diastolic pressure in a very short time.[40] It has been reported that bradykinin, which occurs

in treatment with angiotensin-converting enzyme inhibitors, causes vasodilation by stimulating the release of NO from the endothelium.[41] It has been stated that the response to vasoconstrictor decreases due to the decrease in the endothelium-derived relaxation factor in the blood vessels of diabetic animals and in the pulmonary arteries isolated from people who have undergone heart-lung transplantation.[4]

Under normal conditions, L-arginine methyl derivatives (L-NMA, L-NAME, and others) are excreted in the urine, whereas in cases of acute renal failure, an increase in plasma concentrations has been detected, and hypertension and leukocyte dysfunction have been reported in these conditions.[42] In addition to L-arginine methyl derivatives, chemical agents such as imidazole derivatives and aminoguanidine are also known to inhibit NOS.[43] It has been reported that inhalation of 13-36 ppm NO_2 for seven days can prevent pulmonary hypertension in some lung patients.[33] These results suggest that NO inhalation helps to improve lung function disorders. It has been found that inhaled NO improves the ventilation/perfusion ratio by expanding the capillaries, particularly in the lung alveoli.[44] It is known that inhaled NO has a local effect only in the lungs and does not cause any effect on systemic circulation. It has been stated that under normal conditions, people expel NO at the level of 5-20 ppm during respiration.[33]

THE PHYSIOLOGICAL ROLE OF NITRIC OXIDE IN THE CENTRAL NERVOUS SYSTEM

The discovery of a NO-like substance in the brain in 2003 brought NO to the agenda as a new neuronal mediator.[47] By N-methyl-D-aspartate, which affects the specific glutamate receptor stimulated brain cells synthesize EDRF-like structures.[23] One of these structures is NO.[45,46] In studies, the existence of an active arginine-NO pathway in brain tissue has been proven.[26,47] Nitric oxide shows its physiological effect on the brain tissue by affecting these receptors and increasing the cGMP concentration in the cell. It has become clear that NO plays a role as an eleven neurotransmitter in the sensory pathways induced by glutamate.[22]

NITRIC OXIDE AND LIVER

The liver is an organ with extremely complex and important functions, which are related to all metabolic systems of the organism. The liver engages in various essential functions, including the synthesis of endogenous amino acids and albumin, the production of bile acids, and the regulation of numerous factors involved in blood coagulation. It also produces heparin, a substance that prevents coagulation and serves as a storage site for glucose in the form of glycogen. Additionally, the liver stores certain vitamins, facilitates the detoxification of metabolic byproducts, secretes bile, and contributes to the elimination of harmful microbial agents that enter the body from external sources. It has vital functions such as removing. Disruption of one or more of these functions makes the life of the organism impossible.[47]

Nitric oxide is an important regulator of liver function. The relationship between NO and the liver was mentioned for the first time in studies on cirrhosis. In cirrhosis, physiological events such as NO release, portal system rims, Kupffer cell dispensation, and elimination of intestinal-origin bacterial lipopolysaccharide and endotoxins are carried out by iNOS-containing cells.[48] Nitric oxide, which is released in this way, plays an important role in the basis of hemodynamic disorders in cirrhosis.[49]

Researchers have shown that NO reduces vascular resistance and response to vasoconstrictors in both humans and animals.[48,49] As a result, hyperdynamic circulation occurs, resulting in enlargement of peripheral vessels, an increase in cardiac output, and tachycardia. This event causes the activation of the sympathetic system by decreasing the plasma volume.[50,51] This neurohormonal activation causes water and salt retention in the kidney and creates edema. This situation leads to the redistribution of renal blood flow and leads to the development of hepatorenal syndrome.[52] Hyperdynamic circulation was detected in 30-50% of cirrhotic patients and in all patients with portal hypertension with cirrhosis. It has been reported that the amount of cGMP in the urine increases as an indirect indicator of NO.[33] A study demonstrated that NO, a vasodilator, exhibits an elevation in individuals with liver cirrhosis. This heightened presence

was identified as a significant contributor to the onset of conditions such as ascites and systemic hypotension, which are frequently observed in patients with cirrhosis.[53]

Nitric oxide also causes a decrease in liver protein synthesis, and Kupffer cells are held responsible for this event. Endotoxins activate Kupffer cells that synthesize the iNOS enzyme, causing both NO production and TNF and IL-1 release.[54]

Nitric oxide released in Kupffer cells directly inhibits protein synthesis in hepatocytes. On the other hand, cytokines such as TNF and IL-1 released from Kupffer cells stimulate iNOS in hepatocytes and lead to NO synthesis. Inhibits hepatocyte and Kupffer cell-derived NO protein synthesis. This reversible inhibition occurs a few hours after the stimulus and continues for up to 12 hours. Although the mechanism of this inhibition is not known exactly, it is thought that cGMP alone does not play a role in this inhibitory effect of NO. It is thought to have this effect by inhibiting the translational or post-translational enzymes involved in protein synthesis.[55] In a study on the subject, it was stated that the inhibition of protein synthesis can be abolished with L-arginine analogs that competitively inhibit NO.[53]

In the hyperdynamic phase of cirrhosis, the concentration of endotoxin in the blood increases, the response to vasoconstrictors decreases, and excessive vasodilation is observed. Due to the stimulation of NOS by the present endotoxin, there is an overproduction of NO synthesis. Increased serum levels of nitrate and nitrite, the oxidation products of NO, are observed in cirrhosis, particularly hepatorenal syndrome. It has been reported that this increase is directly related to the endotoxin concentration.[13,56]

NITRIC OXIDE AND THE GASTROINTESTINAL SYSTEM

The increase in saliva secretion and stomach acid during chewing stimulates the release of NO from the stomach. Nitric oxide suppresses acid secretion by inhibiting histamine secretion and also plays a protective role on the gastric wall by increasing mucous secretion.[57]

It is possible to examine the effect of NO on the gastrointestinal system in three groups: neurotransmitter vasodilator and paracrine mediator. It has been reported that during the activation of the NOS enzyme detected in the gastric mucosa, NO is an endogenous vasodilator, and as a result, blood flow in the mucosa increases.[58] In addition to autonomic nerves that strengthen contractions in the gastrointestinal tract, there are neurons with motor and inhibitory functions that suppress contractions. While the mediators of the non-adrenergic, non-cholinergic (NANC) system remain not fully understood to date, it is hypothesized that adenosine triphosphate (ATP) and vasoactive intestinal peptides (VIP) could serve as primary mediators. However, in a study conducted to determine the effects of ATP and VIP, it was observed that the NANC system was inhibited by the administration of L-arginine analogs and oxyhemoglobin, and it was therefore concluded that these substances would not be inhibitors.[59]

NITRIC OXIDE AND THE KIDNEYS

In order for the kidney to maintain its normal function, NO is required in the renal arteries, glomeruli, and tubules. Nitric oxide helps regulate renal autoregulation, tubuloglomerular feedback, renin release, sodium reabsorption, renal blood pressure, and tubular processes. A study discovered a notable decrease in plasma NO levels in cats afflicted with chronic renal failure.[60] Nitric oxide has many effects on kidney functions. However, it is not yet known whether there is any increase or decrease in endogenous NO production in patients with chronic renal failure. However, a recent study stated that NO regulates renal function by regulating vascular tone and sodium reabsorption.[61] Cyclosporine has been reported to decrease NO production in animals with experimental kidney failure and this causes defective construction.[62]

NITRIC OXIDE AS A CYTOTOXIC AND CYTOSTATIC AGENT

Macrophages activated by interferon or bacterial lipopolysaccharides synthesize large amounts of NO. In the absence of activation, NOS was found to be absent in macrophages. It has been reported that NOS synthesis, hence NO synthesis, occurs after macrophage agents are activated. However, excessive NO synthesis can

cause quite a lot of damage to cells. Nitric oxide is formed due to the activation of macrophages; it has cytotoxic effects on bacteria, parasites, and tumor cells.[63]

Nitric oxide exhibits cytotoxic effects on various pathogenic agents, including bacteria, parasites, and tumor cells. This occurs through the inhibition of key processes such as oxidative phosphorylation (specifically ubiquinone reductase), glycolysis (glyceraldehyde-3-phosphate dehydrogenase), and the tricarboxylic acid cycle (involving cis-aconitase and certain Fe-containing enzymes), all crucial for ATP formation. Additionally, NO exerts effects by deaminating DNA.[41] In addition, NO creates an antiviral effect by inhibiting the replication of some viruses.[64] It is stated that the L-arginine-NO pathway in macrophages, particularly monocytes, is an important defense mechanism against tumor cells, and intra and extracellular microorganisms.[65]

THE ROLE OF NITRIC OXIDE IN IMMUNITY AND INFLAMMATION

Several years ago, researcher Fehleisen demonstrated that certain bacterial products enhance resistance to cancer in a manner that is not specific.[66] Researchers showed that some bacterial products could increase resistance to cancer by a nonspecific mechanism.[54,67] According to our current knowledge, this increase in resistance is a non-specific immune event directly related to macrophage activation and NO synthesis. Non-specific immune reactions; It consists of macrophage activation, induction of NOS, and prolonged and abundant NO synthesis. This nonspecific immunity is not limited to the reticuloendothelial system but has also been detected in hepatocytes and lung cells.[68] The role of the liver and lung in NO-dependent non-specific immunity is due to the functioning of these two organs as immunological filters of the circulation.[69] In acute and chronic inflammatory conditions, NO plays an important role by stimulating the production of some proinflammatory substances, eicosanoids, and increasing the effects of cyclooxygenase 1 and 2.[69] Overproduction of NO by macrophages can lead to significant damage to both macrophages and other cells. When macrophages are in an active state, it has been observed that iNOS is inhibited, resulting in a decrease in NO synthesis.

In conclusion, in synthesis, NO, a transient and highly reactive free radical with a nitrogen monoxide structure, assumes a pivotal role in intercellular communication. Its inherent lipid solubility facilitates seamless membrane penetration, culminating in the relaxation of vascular smooth muscle cells upon release from the vascular endothelium. Originating from L-arginine through the catalytic action of NO synthase enzymes, NO serves as a vasodilator and platelet aggregation inhibitor, activating soluble guanylyl cyclase and elevating cGMP levels. The EDRF, functioning as a secondary messenger, extends its regulatory influence across diverse cellular contexts. This concise overview underscores the structural, biosynthetic, and physiological significance of NO in the realm of cellular health.

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