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NITRIC OXIDE AS A BIOPHYSIOLOGICAL COMPONENT and A RADICAL METABOLITE

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Bir biyofizyolojik eleman ve radikal olarak azot oksit.

ABSTRACT

A wide amount of reactive oxygen species and other free radical oxygen metabolites are produced in the body, food systems and in the environment. Free radicals and most of the reactive oxygen metabolites are chemical agents that have a single unpaired electron. Nitric oxide made up of a nitrogen and oxygen atom is one of the smallest molecules. Until recently it has been known as a gas produced by combustion of fuel inside engines and emitted to air polluting and damaging the ozone layer. Increased levels cause acid rains. On the other hand it may play important roles in the human body. Recently, much interest has been focused on free radicals and it has been shown that NO is a free radical. NO is formed in vivo from the amino acid L-arginine. N02 is formed when NO-radical reacts with 02, and is found in polluted air and smoke from burned organic materials. It is known that NO has significant biological roles and participates in large amount of pathological processes as a free radical. This review describes how NO is formed and possibly possible participated in the basic reactions, and its role in human physiology and pathophysiology.

KEY WORDS: Nitric oxide, free radicals, oxidant stress, reactive species.

ÖZET

Organizma, gıdalar ve çevre reaktif karakterdeki pek çok oksijen metabolitini üretmekte ve içermektedir. Serbest radikal madde olarak tanımlanan bu atom ve bileşikler özellikle hücrelerde membran lipitleri peroksidasyonu, protein yıkımı ve DNA mutasyonlarına yol açarak geri dönüşmesi imkansız hücre hasarları oluşturmaktadır. Uzun yıllar bir nörotransmiter madde olarak tanınan azot oksitin bir serbest radikal olabileceği ileri sürülmekte, bir radikal olarak nasıl aktivite gösterdiği üzerinde yoğun çalışmalar sürdürülmektedir. Asit yağmurları ve ozon katmanını haraplayıcı etkileri ile bir çevresel kirletici ajan olarakta bilinen NO'nun biyolojik bir radikal ürün olduğu kanısı yaygınlaşmaktadır. Serbest radikallerin verimllik, performans, hastalıklara karşı direnç ve sağlıklı bir yaşam sürdürülmesinde önemli oranda etkili oldukları bilinmektedir. Azot oksit (NO), bir oksijenle azot atomundan oluşan ve azot oksit sentaz enzimince sentezlenen , organizmadaki en küçük moleküler yapılardan biridir. Derleme NO'nun kaynakları, üretilmesi, reaksiyonları, fizyolojik ve patolojik olgulardaki rollerinin ele alındığı güncel çalışmalar ve kanıları araştırmacıların dikkatine sunmak amacıyla hazırlanmıştır.

ANAHTAR KELİMELER: Azot oksit, radikal, oksidasyon

INTRODUCTION

Free radicals contain one or more unpaired electrons and can be monatomic such as halogen atom Br, Cl, alkali metals like Na, K, or transition metal ions like Cu and Fe. Some atomic combinations are entitled as radicals that leave a single electron such as nitric oxide (NO), nitrite dioxide ($N0_2$) and dioxygen (O_2). The electronic structure of NO, NO_2 and O_2 are shown below.

•NO::N O::N = O .OO.

Nitric oxide has an unpaired electron than reacts rapidly with dioxygen, nitrogen dioxide, thiol groups, and superoxide. This makes the molecule highly reactive. In the cells it readily reacts with molecules such as oxygen, superoxide and transition metals like Fe, Cu and Mg (Vanin 1993, Zhang 1993). Therefore, its half life reported to be 3-5 s in vivo approximately (Hibbs 1988, Ignore 1990, Michael 1995).

Recent studies have shown that many mammalian cells synthesize nitric oxide from the amino acid L-arginine in vivo (Hibbs 1988, Michael 1995). The proposed mechanism for the synthesis of .NO involves the oxidation of L-arginine to NO and L-citrulline(Ignore 1990). The reaction also requires an electron donor, such as nicotinamide adenine dinucleotidephosphate (NADPH), and the cofactor tetrahydrobiopterin (H_4BPT). During the synthesis of NO, the removal of the nitrogen atom from arginine produces the amino acid sitrulline.

$N0_2$ is formed when NO reacts with 0_2, and is found in polluted air and smoke from burned organic materials. NO is very reactive against the oxygen. It can easily be inactivated without requiring a special enzyme. Very fast reaction of superoxide with NO radical gives non radical peroxynitrite and reaction with 0_2 gives NO_2 or $N0_3$ forms which are less active products (Halliwell 1995, Zhang 1993).

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

$O_2 + NO \rightarrow ONOO^-$ (peroxynitrite)

Like other reactive oxygen metabolite reproducing mechanisms, cells produce NO and sometimes it may go awry. As a consequence, NO has been implicated in a number of diseases and disorders like other radicals. These range from septic shock, diabetes mellitus, cardiac disorders to Alzheimer's dementia and gastric ulcers.

PHYSIOLOGICAL IMPORTANCE of NITRIC OXIDE

The significance of chemical effects of NO in organism was nowhere more aptly demonstrated than in the experience of Lancaster (1992). Having discovered the pleasing effects of nitrous oxide (N_2O) commonly called laughing gas, Davy were nearly died when he inhaled small amount of NO. Although structurally similar, the two oxides of nitrogen have very different physiological effects. Nitrogen compounds are disposed from the human body by urine. Until recently they have been thought to be derived from ingested foods. However some investigations led to suggestions that propose production of nitrates within the cells like other free radical species. For example, human and rat fed with low nitrate containing diet were still found to be excreting normal amounts of nitrates (Padmaja 1993).

15-20 years ago it was shown that the source of nitrates were macrophages. Although it was known that the macrophages destroyed infected cells by using NO and superoxide (O_2^-), some studies reported that the same method was in attack against the tumour cells (Holmquist 1993, Padmaja 1993, Pollock 1994). It was also put forward that they produced nitrogen compounds by stimulation of cytokins released from Tlymphocytes (Holmquist 1993). This microbicidal activity of nitric oxide can be seen in several kinds of infections. Some studies even reported that pathological and destruction capacity of some micro-organisms were parallel to their resistance against nitric oxide as in Mycobacterium avium (Mayer 1992, McMillan 1993, Merrit 1993) Studies about the destructive effect of NO presented that molecule exerted it's toxic effect by binding to certain enzymes acting in cell respiration and preventing their function. Description of the fact that methyl arginine inhibits the nitric oxide synthetase (NOS), and several researches concerning the fact proved that NO has rather important roles in the immune system (Holmquist 1993, Merrit 1993, Padmaja 1993).

It was also reported that NO had an important role in graft rejection after transplantation (Person 1993).

CARDIOVASCULAR and MUSCULAR EFFECTS of NITRIC OXIDE

The muscle layer of the endothelial cells regulates the blood pressure by contractions and relaxation. While acetylcholine causes relaxation norepinephrine makes just the opposite. Since norepinephrine receptors were detected on muscle cells, acetylcholine receptors were also thought to be in the some localization. At the beginning of 80's it was shown that this was not just true. When endothelial layer was removed, acetylcholine was unable to produce its relaxing action. In a series of studies, it was investigated that acetylcholine receptors are on endothelial cells. It was also reported that when acetylcholine acted on endothelial cells, a small molecule was released and it caused muscle relaxation. It was referred to endothelial derived relaxing factor (EDRF) (Holmquist 1993, Holscher 1993). But determination of EDRF was almost impossible. Lots of author tried to isolate the molecule but non of them could active. However, in some authors reported that EDRF activated cyclic guanosin monophosphate (cGMP) as a secondary messenger. Mean while, the mechanisms of nitro-glycerine action were studied, and it was found that organic nitrates were in fact inactive but they caused relaxation when converted to nitric oxide. Consequently, EDRF and nitro-glycerine studies unified at a common point. Relation between EDRF and NO was tried to be cleared off by examining the reactions, the two substances give rise to. Like EDRF, NO had a very short half life in oxygen-rich environment. The function of EDRF could be inhibited by superoxide anion and by haemoglobin molecules with which NO also reacts rapidly. It was referred that endothelial cells cant produce EDRF without argentine, (Holmquist 1993, Holscher 1993, Salazar 1993, Wang 1993).

Before the effects of NO on muscles were understood, an unstimulated vessel was thought to be relaxed. However, it was found that vessels constricted without stimulation. Only in the presence of relaxing factors blood pressure could be controlled and this was principally due to the action of NO (Stevens 1993, Trigo 1993). Therefore, regulation of blood pressure does not depend on mainly angiotensin and norepinephrine as previously accepted, but on the activity of NO (Kroncke 1993, Kwiatkowski 1992).

Today, it is known that the functions of the NO in cardiovascular system is not limited to relaxation of blood vessels, number of other actions such as those in thrombocyte aggregation, control of anal sphincter, penile erection etc. (Rockett 1992, Roberts 1994).

Table 1. Physiological role of NO in cardiovascular and muscular system (Hollan 1995)

NO. (or derivatives)	→ smooth muscle cell relaxation
O_2^- + NO	→ $ONOO^-$ (peroxynitrite) vasoconstriction regulation of vascular tone

NITRIC OXIDE IN NERVOUS SYSTEM

After learning the effects of NO in immune and circulatory systems, it was traced also in brain and neurones. The first clues showing that this radical played role in nervous system appeared when arginine was found to be essential for cGMP synthesis in the brain, fifteen years ago. At that time, NO was not known as a transmitter and necessitated arginine for production. In 1989 the

role of NO radical in synaptic stimulation was started to be studied. The next stage was determination of action area of NO in the brain. Since the molecule had a very short life span like other radical species, not NO, but NOS was aimed to be determined (Akkuţ 1996, Holmquist 1993).

Investigations about the relationship between NO, Ca++ and calmodulin clarified the mechanism by which NMDA receptors accelerated the NO synthesis. As we know, glutamate increases the Ca++ influx into the cell by binding to NMDA receptors and Ca++ ions inside the cell activatps NOS by attaching to calmoduline which happens in few milliseconds (Berner 1993).

NOS has been observed in different neuronal groups. In pituitary gland for example, enzyme is merely found in neurones whose cells bodies are located in hypothalamus. These special neurones are responsible for secretion of vasopressin and oxitocin. NOS has been detected in nerve plexus which induces adrenal cells to secrete adrenaline in suprarenal glands. In the intestines, the enzyme is in miyenteric plexuses. Although NO is traced in whole brain, available data did not suggest any fact about its functions at the beginning (Azadaoi 1992, Berner 1993).

NO AS A NEUROTRANSMITTER

Nitric oxide has a very specific distribution in the nervous system, different from all other neurotransmitters (Holmquist 1993, Holscher1993). Therefore NO is accepted an unusual molecule as a transmitter. Neurotransmitters are normally stable molecules and stored in vesicles. NO-radical is not found in cytoplasm, but synthesized when a stimulation arises. NO leaving a cell by simple diffusion, enters an other cell again by diffusion without binding to receptors on postsnaptic cell membrane. Its target is Fe+ atoms in active side of cGMP producing enzyme. NO; binding to Fe+, enhances the activity of the enzyme by changing its 3-dimensional configuration. NO represents a new kind of neurotransmitter with special action mechanism. Recent studies have shown that NO acts as a new transmitter in non-adrenergic, non-cholinergic inhibitory nerves of human and animal airways (Chapman 1992, Doi 1993).

NITRIC OXIDE IN DISEASES

Nitric oxide and other free radical species are generated normally by aerobic metabolism in cells and this generation can significantly increase in certain pathologic conditions (Aslan 1995). The increased radicals damage the cells and cause the diseases. Roles of NO-radical in several diseases have recently been understood.

NO obviously seems to be of vital importance in virtue of its roles in cellular functions (Finberg 1993, Halliwel 1995, Hibbs 1988, Michael 1995). However, it sometimes appears to be not only in brain and nervous system but in other organs as a free radical damaging the cells and tissues. One of these instances is septic shock. We face to NO-radical as a causative agent in pulmonary vascular tonus impairment with hypotension seen in septic shock. Related to this, it has been demonstrated that bacterial endotoxines induced the NO synthesis in endothelial cells and thus by its vasodilator property reduced the blood pressure. Bacterial endotoxines appear to be causing release of a substance by macrophages damaging hepatocytes. Several studies have reported that this substance is NO-radical (Durieu 1993, Finberg 1993, Hollan 1995, Zhang 1993). Again it has been suggested that considerable improvement had been observed in patients with sepsis with treatment of arginine analogies, inhibitors of NO-radical synthesis (Finberg 1993). The important role of NO in the complications of diabetes mellitus (DM) have also been demonstrated in several researches. It has been proposed that, insulin dependent diabetes mellitus may be etiologically due to the destruction of the pancreatic islet cells by macrophages considering a foreign tissue. Interleukin-1, released from immune system cells, causes the pancreatic tissue to produce high amount of NO-radical and hence damage itself (Holmquist 1993). Some experiments have reported that arginine analogies have proved that NOS inhibition may be effective in prevention of pancreatic tissue damage (Holmquist 1993). Somehow increased NO-radical production playing a role on initiation of diabetic vascular pathologies. Failure of penile erection, another complication of DM is also attributed to a mechanism related with NO-radical production disorders (Keller 1993, Stief 1992).

Partial role of NO-radical in the etiology of portal hypertension has been investigated. It has also been demonstrated that NO has natriuretic property beside its vasodilator action (Girard 1992, Lancaster 1992). Pathological effect of NO-radical in central nervous system (CNS) is an area to be studied itself. The molecule participates in the etiology of some degenerative neuronal disorders like Alzheimer's disease. Especially its relation with long term potentiation gives idea about the cell injury seen in Alzheimer's disease (Rebeck 1993). Concerning neuronal damage it has been proposed that microglias activated by some factors such as hypoxia, hyperoxia and mechanical injury. Brain macrophages secreting substances like cytokines and reactive nitrogen compounds are responsible for the pathology (Battafarano 1992).

Roles of the NO in demiyelinating autoimmune diseases have been studied. In mice with allergic encephalomyelitis, especially multiple sclerosis, NO radicals in complex with iron have been detected. These investigations prove that NO plays an important role in demyelinating diseases and especially in multiple sclerosis (Andrade 1993, Bohrer 1993).

Induction of enzyme by some cytokines produced by immune cells such as neutrophils and macrophages participates in the etiology of inflammatory bowel disease. NO-radical causes cell injury, intestinal hyperaemia and intestinal smooth muscle dysfunction. In addition, carcinogenic nitrosamines are formed on decomposition of NO in solutions. Therefore it is claimed that antioxidants preventing formation of nitrosamines can also alter the carcinogenic agents to be formed (Holmquist 1993).

CONCLUSIONS

NO and other reactive metabolites are formed in vivo naturally. Their formation increases in most human diseases. There is much to learn from the role of NO in the cell. This is the importance of chemistry to the understanding of basic physiological processes. The tight link between nitric oxide's structure and its actions is apparent not only in the everyday activities of the body mass, but also in the processes of disease. The term nitric oxide provides an example of the increasing utility of chemistry in solving the problems of medical sciences. NO and other radical-induced damage occurs constantly in the human body and has to be repaired. Most studies in which NOS inhibitors are given to human body and animals presented several cardiovascular changes, mainly a significant increase in blood pressure. Modification of NO-metabolite secretion by certain agents may give rise to new treatment modalities for some disorders also. As a result NO is a molecule which importance, having considerable physiological significance and to may cause a series of pathologies has yet been understood. Thus it is obvious that studies to be done about this radical/molecule may bring about new physiological insights.

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