

CONFERENCES AND SYMPOSIA

PACS number: 01.10.Fv

Scientific session of the Division of General Physics and Astronomy of the Russian Academy of Sciences (22 December 1999)

A scientific session of the Division of General Physics and Astronomy of the Russian Academy of Sciences (RAS) was held on 22 December 1999 at the P L Kapitza Institute for Physical Problems, RAS. The following reports were presented.

(1) **Vanin A F** (Institute of Chemical Physics, RAS, Moscow) "Nitric oxide and its detection in biological systems by the electron paramagnetic resonance technique";

(2) **Stepanov E V, Milyaev V A** (Institute of General Physics, RAS, Moscow), **Selivanov Yu G** (P N Lebedev Physics Institute, RAS, Moscow) "Laser orthomolecular medical diagnostics";

(3) **Rozanov N N** (Institute of Laser Physics, S I Vavilov State Optical Institute, St Petersburg) "Dissipative optical solitons".

An abridged version of the reports are given below.

PACS numbers: **87.64.–t**, 87.64.Hd, **87.80.+s**, **87.90.+y**
DOI: 10.1070/PU2000v043n04ABEH000719

Nitric oxide and its detection in biological systems by the electron paramagnetic resonance technique

Vanin A F

Biologists, biochemists, and biophysicists have recently been faced with an unexpected fact. It has turned out that animal and human cells and tissues contain a very simple compound, a two-atomic molecule of nitric oxide (NO), which can function as an endogenous regulator of metabolic and physiological processes alongside bigger molecules including proteins. Before this fact emerged, it had been universally accepted that highly selective bioregulation is feasible only through the specific interaction between chemical regulators (signal molecules, according to the current usage) and corresponding receptors on the cell surface. Such selective interaction, which is indispensable for an extracellular signal to be taken up by the cell, is believed to be mediated through the specific spatial structure of a signal molecule complementary to the molecular structure of the receptor, specific electron density distribution on the signal molecule, its ability to change these parameters upon binding to the receptor, etc.

Nitric oxide, as a signal molecule, influences intracellular processes without interacting with receptors. It undergoes diffusion across the plasma membrane and interacts with target molecules inside the cell. How does this simple compound with such a rigid spatial and electronic structure selectively trigger intracellular metabolic processes? This question remains to be answered. It appears from the general considerations that the selectivity of NO action is achieved by virtue of the high chemical affinity of this molecule for its major target, the active centre or other functional group of an enzyme. The tight binding of NO to such a target either stimulates or inhibits enzyme activity and thereby modulates respective biochemical processes. Non-specific effects of NO, which is a highly reactive agent, on other intracellular systems are probably prevented by its rapid oxidation (e.g. by superoxide anions) which leads to the formation of products showing low biological activity, largely nitrites and nitrates.

Nitric oxide functions as a signal molecule in practically all organs and tissues of animals and man. It is continuously produced in these structures by means of enzymatic synthesis involving the so-called NO-synthase which uses L-arginine as the sole amino acid substrate. NO-synthase-catalyzed oxidation of the amino group in the guanidine residue of L-arginine results in the release of a free NO molecule while L-arginine undergoes conversion into another amino acid, L-citrulline.

To date, the best known biological effects of NO include relaxation of blood vessels mediated through the inhibition of adrenaline and other vasoconstrictors. In case of deficient NO synthesis in blood vessels, they tend to constrict rather than dilate which accounts for vasospasm, impaired passage of blood, high blood pressure, and concomitant pathological changes. These disorders may be corrected by such pharmaceuticals as organic nitrates of which the best known is nitroglycerin. The beneficial effects of this drug and other organic nitrates are due to the production of nitric oxide which causes relaxation of blood vessels and thus eliminates vasospasm. This action of organic nitrates was first described quite recently, i.e. almost 150 years after nitroglycerin came to be used as a medicine.

The spasmolytic effect of endogenous nitric oxide and medications producing it results from a series of biochemical processes which ends in a surge of calcium ions from smooth vascular cells. This, in turn, causes vasodilation. NO triggers these processes by activating a most important cellular enzyme, guanylate cyclase. The high affinity of NO for the heme group of this enzyme accounts for the tight NO binding to the iron atom and the resulting change in enzyme conformation is responsible for its acute activation. Cyclic guanosine phosphate, a product of the catalytic activity of guanylate cyclase, triggers a series of biochemical processes leading to the relaxation of blood vessels.

Synthesis of NO from L-arginine continuously occurs in the central and vegetative nervous systems. In the former, this agent is indispensable for the establishment of long-termed

Uspekhi Fizicheskikh Nauk **170** (4) 455 – 465 (2000)
Translated by Yu V Morozov; edited by M S Aksent'eva

links between neurons which are known to be involved in the mechanisms of memory, learning and, therefore, human creative activity. NO synthesis in the vegetative nervous system ensures its regulatory action on the gastro-intestinal tract and uro-genital system. NO acts as a signal molecule in secretory tissues and respiratory organs. The biological activity of skin tissues also depends on NO.

The regulatory functions of nitric oxide are manifest at its steady-state tissue concentrations of several micromoles per kilogram. At a higher production rate (up to tissue concentrations of 100 μM kg^{-1}), nitric oxide exhibits cytostatic/cytotoxic activity and may function as an effector of the cellular immunity system that protects the organism from bacterial infections and carcinogenic influences.

Enhanced nitric oxide production occurs not only in immuno-competent cells but also in vascular smooth muscles, myocardium, nervous and secretory tissues. It may be responsible for a variety of pathological changes. For example, increased NO synthesis in blood vessels may lead to endoseptic shock associated with a sharp decrease in peripheral vascular resistance caused by excessive vasodilation with a rapid irreversible fall in blood pressure. This process is initiated by biologically active compounds produced by endogenous bacteria or body tissues in response to bacterial infection. Another example is stroke development induced by activation of neuronal NO synthesis under hypoxic conditions or in case of nutrient deficiency. Excess NO and its oxidation products are transferred from these neurons to the neighbouring nerve cells and kill them. The two pathologies, i.e. endoseptic shock and stroke, can be prevented by the timely suppression of intracellular NO synthesis using specific inhibitors.

In biology, intense interest in NO arose less than 10 years ago. Before, most biologists considered nitric oxide to be a biologically and ecologically 'harmful' agent. A breakthrough in this problem was heralded by the works of the American physiologist and pharmacologist Ferid Murad and his group. In the 1970s, they demonstrated that NO has beneficial biological effects; specifically, it activates guanylate cyclase, one of the most important regulatory enzymes. The mechanism of such activation was described in previous paragraphs. Studies carried out by F Murad and co-workers promoted the understanding of the cause of hypotensive, spasmolytic, and antithrombotic actions of various nitroso- and nitro-compounds including nitroglycerin. This action is attributable to their ability to produce NO. Interest in the biological role of NO was further stimulated by the discovery of the so-called endothelium-derived relaxing factor (EDRF) by the American physiologist Robert Furchgott in 1980. This agent was shown to be released by endothelial cells lining the blood vessels under the action of hormones and other biologically active compounds. R Furchgott and another American physiologist and pharmacologist Luis Ignarro were the first to show that nitric oxide is an active component of EDRF and that it is responsible for the vasodilating effect of EDRF.

In 1998, F Murad, R Furchgott, and L Ignarro shared the Nobel Prize for Medicine or Physiology for these works.

In addition, the American researchers J Hibbs, M Marletta, and D Stuehr made a new important contribution to the development of nitric oxide research in the 1980s. They observed NO synthesis by activated macrophages and showed that it was responsible for their cytostatic/cytotoxic action. A study on the NO role in nervous tissue was initiated by J Garthaite (UK), S Snyders and O Bredt (USA) in the late 1980s. As early as the 1960s, Russian researchers (A Vanin, A Saprin) used electron paramagnetic resonance (EPR) to detect paramagnetic nitrosyl complexes of heme and non-heme iron in the human and animal body. This discovery may be regarded as the first evidence of the existence and production of NO in living systems.

The discovery of NO in biological objects was delayed by the absence of reliable methods for its direct detection in these systems. NO being a highly reactive compound, its practically unhampered interaction with superoxide ions accounts for the rapid depletion of this compound. As a result, the steady-state NO concentrations in living system remain rather low (at the micromole level). The problem was resolved in our laboratory in the 1980s when highly effective NO traps were first proposed. These ensured its accumulation in the animal body and cell cultures over 1 – 2 hours with the subsequent measurement of the available amount of nitric oxide. The traps were actually complexes of divalent iron with dithiocarbamate derivatives. Their binding to NO produced paramagnetic mononitrosyl iron complexes (MNIC) which were identifiable by the EPR technique. These complexes were found to be associated either with membranous (hydrophobic MNIC) or aqueous (hydrophilic MNIC) cellular fractions. MNIC containing diethyldithiocarbamate (MNIC – DETC) and N-methyl-D-glucamine-dithiocarbamate (MNIC – MGD) became the most extensively used hydrophobic and hydrophilic MNIC respectively. An unpaired electron delivered to these complexes by an NO molecule is further transferred onto the iron atom which results in the localization of a total of 7 electrons on its d-orbitals (d^7 electronic configuration), with the concomitant conversion of NO into NO^+. The unpaired electron is preferentially located in the d_z^2 orbital of the iron atom. The general formula of such complexes is $Fe^+(NO^+)(S_2CNR_2)_2$.

In principle, NO can just as well bind to complexes of dithiocarbamate derivatives with trivalent iron. MNIC resulting from such interaction are diamagnetic and therefore can not be detected by EPR. However, these diamagnetic MNIC undergo rapid transformation to the paramagnetic form especially in hydrophobic media. The transformation occurs through reductive nitrosation — the one-electron reduction of Fe^{3+} to Fe^{2+} with nitric oxide. The resulting ion NO^+ binds to molecules of the solvent while its coordination site in MNIC is occupied by another neutral NO molecule.

Signals from MNIC – DETC and MNIC – MGD recorded by EPR have identical shape and parameters. They are characterized by the approximately axially-symmetric tensor of the g-factor with $g_\perp = 2.04$, $g_\parallel = 2.02$ and the permitted triplet hyperfine structure (HFS) at $g_\perp$. If the signals are recorded at room temperature, averaging of the anisotropy of the g-factor and HFS leads to the detection of an EPR isotropic signal with the centre at $g = 2.04$ and isotropic triplet HFS ($12G$ splitting). This HFS arises as a result of the interaction between the unpaired electron density and the ^{14}N nucleus with spin $I = 1$. The substitution of ^{14}NO by ^{15}NO (nuclear spin of ^{15}N equals 1/2) leads to doublet HFS. Additional doublet splitting of the EPR signal of MNIC in HFS may also occur if ^{56}Fe ($I = 0$) is substituted by ^{57}Fe ($I = 1/2$).

The use of the nitrogen isotope ^{15}N in conjunction with the proposed NO traps allowed for the identification of NO sources in animals. It turned out that the administration of

L-arginine, in which is the amino group of the guanidine residue labelled with ^{15}N, resulted in the formation of MNIC which exhibited the doublet HFS in the absence of triplet structures. This gave unambiguous evidence that in animals NO is synthesized only from L-arginine. Experiments in which the oxygen isotope ^{17}O was utilized showed that HFS changed due to the hyperfine interaction with the nucleus of this isotope. It was inferred that atmospheric oxygen is involved in oxidation of the amino group in the guanidine residue of L-arginine to NO. Incorporation of ^{57}Fe into MNIC–MGD was used to trace the transformation of these complexes in mice. It was demonstrated that almost all of them are excreted with urine within one hour after administration. In contrast, hydrophobic MNIC–MGD are retained in different organs.

The EPR spectroscopic technique we proposed for the detection and quantitation of NO is now extensively used worldwide to study NO in biological systems. Moreover, it provided a basis for the development of EPR tomographic analysis in animal tissues at the laboratories of J Zweier (J Hopkins University, Baltimore, USA) and T Yoshimura (Institute for Technological Life Support, Yamagata, Japan).

In addition to the incorporation of NO into exogenous iron-containing traps, it can form in cells and tissues paramagnetic dinitrosyl iron complexes (DNIC) composed of endogenous iron and thiol (sulfur-containing) groups of proteins and low molecular weight compounds. We first found such complexes in yeast cells (1963) and thereafter in animal tissues (1967). They were identified by recording EPR signals from the cells and tissues characterized by axially-symmetric tensors of the g-factor with $g_\perp = 2.04$, $g_\parallel = 2.014$, $g_{av} = 2.03$ (2.03 signal). The nature of the centres responsible for these signals was elucidated after we found a paramagnetic compound the shape and parameters of whose EPR signal was identical with those of the 2.03 signal. This signal was recorded in a frozen DNIC solution with a thiol-containing amino acid, cysteine. Subsequent comparative physico-chemical analysis of these complexes and centres characterized by the 2.03 signal demonstrated the identical nature of the compounds of interest. A specific feature of DNIC synthesized in biological objects is the protein nature of their ligands, i.e. iron contained in these complexes coordinates with cysteine residues of their proteins. In living systems, a fraction of DNIC with low molecular weight thiol-containing ligands is as a rule insignificant.

Analysis of the electronic and spatial structures of the detected DNIC has demonstrated that in these low-spin complexes, as in MNIC with dithiocarbamate derivatives, the unpaired electron is largely localized in the d_z^2 orbital of the iron atom. The geometric structure of the complexes is either octahedron or square, with ligands in the cis-position at the square plane. The iron is in the Fe^+ state (electronic configuration d^7) while NO ligands are in the state NO^+.

Parallel to our studies, B Commoner and his group (USA) discovered and identified DNIC with thiol-containing ligands in rats developing hepatoma induced by various hepatocarcinogens (1965–1970). The complexes arose at the initial stages of tumour growth in the absence of apparent morphological changes in the liver. They disappeared at the later stages. These studies were interrupted until the 1990s but were restarted after the discovery of NO synthesis through the L-arginine pathway in animal tissues and cells (J Hibbs and R Bastian, USA). At present, endogenously produced DNIC complexes are known to occur in different types of cultured animal cells which synthesize nitric oxide.

The functional role of these complexes remains to be elucidated. There is every reason to believe that they may serve as storage depots or a transport form of NO, along with S-nitrosothiols.

Administration of such complexes obtained by chemical synthesis to animals (in the capacity of NO donors) results in different physiological effects. Specifically, they decrease platelet aggregation, reduce blood pressure, and dilate vessels. In other words, they can be utilized as basic materials to develop a new class of cardiovascular drugs. An interesting property of these materials is their ability to induce synthesis of stress proteins including the so-called heat-shock proteins which defend the organism against a variety of stressful factors.

To summarize, a new discipline, NO biology, has set in as a new field of natural science. NO studies yield to-day and will yield in the future new fundamental data which can be applied to practical medicine. The accomplishment of this task will require the concerted efforts of biologists, biochemists, physiologists, and biophysicists working in close collaboration with chemists and physicists.

PACS numbers: 42.62.Be, **87.80.+s, 87.90.+y**
DOI: 10.1070/PU2000v043n04ABEH000720

Laser orthomolecular medical diagnostics

E V Stepanov, V A Milyaev, Yu G Selivanov

A promising field for the application of high-resolution laser molecular spectroscopy is an extremely sensitive analysis of gas exchange in biological objects. This method is applicable to the detection of microconcentrations of relatively light gaseous molecules in the human body formed as endogenous products of its vital activity. The data thus obtained may be used to evaluate health status and diagnose various disorders. When used for medical purposes, the method is usually referred to as orthomolecular diagnostics.

For several reasons, one of the most attractive areas in which to employ orthomolecular diagnostics is microanalysis of the composition of exhaled air. Respiration is the most effective mode of gas exchange between the human body and the environment. Thanks to the large area of the alveolar surface (over 100 m^2), human lungs provide a highly effective gas-exchange system which ensures the utilization of atmospheric oxygen and the disposal of final metabolic products. Apart from CO_2, the major metabolite, exhaled air contains about 600 other volatile compounds formed in the course of numerous biochemical reactions, which need to be released from the body. Both synthesis and transport of individual compounds are based on highly specific mechanisms. This allows them to be used as natural biological markers of the processes which occur in the human body. Moreover, the continuity and cyclicity of respiratory movements are important prerequisites for the continuous and long-lasting monitoring of these processes in close-to-real-time regime. The last but not the least advantage of orthomolecular diagnostics in clinical conditions is that it is a non-invasive method for obtaining valuable information, that is it gives the possibility to make measurements and examine a patient without invasion to studied organism.