Nitric oxide:
From menace to marvel of the decade

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Summary Research papers continue to flood the scientific journals with insights into the biological activity and potential clinical uses of nitric oxide (NO): a gas controlling a seemingly limitless range of functions in the body. Each revelation adds to nitric oxide's already lengthy resume in controlling the circulation of the blood, and regulating activities of the brain, lungs, liver, kidneys, stomach, gut, genitals and other organs.

The molecule governs blood pressure, through a recently recognized process that contradicts textbook wisdom. It causes penile erections by dilating blood vessels, and controls the action of almost every orifice from swallowing to defecation. The immune system uses nitric oxide in fighting viral, bacterial and parasitic infections, and tumors. Nitric oxide transmits messages between nerve cells and is associated with the processes of learning, memory, sleeping, feeling pain, and, probably, depression. It is a mediator in inflammation and rheumatism.

Clinically, newborn babies with breathing problems are getting relief by an experimental inhalation treatment developed from this new understanding. A novel class of drugs that block the production of nitric oxide is being assessed as a possible treatment for septic shock. Drugs that liberate or enhance the action of nitric oxide may be useful in the treatment of pulmonary hypertension, and could prevent the formation of blood clots and counteract impotence. Animal experiments show how to manipulate nitric oxide production to stop the development of arthritis and kidney disease.

In the longer term, drugs that alter the amount or activity of nitric oxide might help protect the brain in conditions such as stroke, Alzheimer's and Parkinson's disease.

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Five years ago the Welcome Institute for the History of Medicine began a series of events called Witness Seminars. They provide a forum for recording for the archive, first-hand accounts of the circumstances leading to major advances in contemporary biomedical science, like the discovery of monoclonal antibodies and unraveling the cause of thalassaemias, a group of inherited blood disorders that are widely spread across the Mediterranean, Middle East and Far East, and cause severe anemia.

They capture the sort of reminiscences and perspectives that could only come from the intimacy of an autobiography of those in the front line of the research.

A similar chance to savour a first-hand narrative of an area of research, though at an earlier stage of science in the making, was provided by Professor Salvador Moncada, Dr Patrick Valance, Professor John Garthwaite and Professor Eddy Liew. They distilled the significance of a welter of basic and clinical research by scores of international laboratories into how nitric oxide, synthesized from the amino acid L-arginine, literally in cells from head to foot, can keep us fit and healthy.

The research is now yielding over 3 000 papers a year. It began with the discovery that the nitric oxide molecule was the regulator of the muscle tone of blood vessels. While the finding astonished some scientists and provoked skepticism in others, it opened a floodgate. The potential importance of the torrent of research results, which showed nitric oxide governed numerous other biological processes, received an accolade guaranteeing wider public attention when nitric oxide was chosen as the cover story by Science for its "Molecule of the Year" in 1992, and was wreathed with puns: NO sex; NO wonder; NO way.

However, it couldn't match the vivid account of "the fascinating adventure" Salvador Moncada lives when recalling the research that unmasked the biological role of nitric oxide and the subsequent avalanche of work it triggered.

He was a senior author of the paper, published in 1987, which revealed how nitric oxide is produced to control the relaxation of the muscles of blood vessels. He clearly recalls the surprise expressed by other researchers when they realized that such a crucial activity, with profound implications for understanding the cardiovascular disease, was under the control of such an apparently simple molecule.

The story had its origins seven years earlier. In effect, the gauntlet was thrown down by R F Furchgott and J V Zawadski who showed that when strips of blood vessels, nurtured in an organ bath, were chemically stimulated, the muscles relaxed. If the inner layer of cells of an artery or vein, the endothelium, was absent, the smooth muscles of the blood vessel lost their capacity to make the vessel expand. This showed that a previously unrecognized substance must exist that regulated the tone of the smooth muscles of blood vessels. They referred to the mystery agent as endothelium dependent relaxing factor, EDRF. Curiosity provoked several laboratories to start searching among the body's complex bio molecules to find a candidate for EDRF among the array of amines, peptides, fatty acids and other chemicals.

Salvador Moncada and his colleagues devised two experiments to test whether nitric oxide could account for the actions of EDRF. The first was to determine whether nitric oxide was released by endothelial cells. Equipment developed for the study included a highly sensitive, miniaturized version of an instrument used in the car industry to measure nitric oxide in the exhaust of petrol engines. When linked to endothelial cells, repeated measurements demonstrated that nitric oxide was indeed the relaxing factor released by these cells.

The second experiment compared the effects of natural EDRF on muscle tone with the effects produced by mimicking the relaxing factor with "off the shelf" nitric oxide, bought from the British Oxygen Company, the specialist that made industrial gases by the ton rather than in the picogram amounts of nitric oxide made by endothelial cells.

Salvador Moncada recalled that nobody had the remotest suspicion that EDRF would turn out to be a simple molecule like nitric oxide; or that it was synthesized not just in the blood vessels, but as an essential part of the physiology of most organs and tissues.

Previously, nitric oxide was regarded as an environmental pollutant and little else: at best a chemically reactive nuisance, at worst a poison. In the exhaust fumes of cars it reacted readily with oxygen to produce smog, increasing the risk of asthma. When discharged into the atmosphere from power station chimneys it contributed to the ecological damage from acid rain. Consequently, a response bordering on disbelief greeted the discovery that cells lining the walls of blood vessels, endothelial cells, intentionally synthesized nitric oxide as a muscle relaxant. The molecule is short-lived, and a constant supply is generated by endothelial cells in response to the sheer stress of the blood flow on the artery wall. The notion that such a noxious little molecule should also hold a key to a healthy body and mind was counter-intuitive, and is still disconcerting to some people.

Compared with the complexity of the hundreds of other molecules that keep us ticking, where size seems to equate with biological relevance, the free radical form of nitric oxide that constitutes the muscle relaxing factor is simplicity itself: just one atom of oxygen and one of nitrogen. However, this seemingly uncomplicated molecule has a number of reactive forms, which helps explain the diversity of its chemistry and the range of biological effects they stimulate.

A year after identifying nitric oxide as the mystery molecule, Salvador Moncada's group reported on how it was formed in endothelial cells. The biochemistry depends on the action of one of a family of enzymes, nitric oxide synthesis or NOS, converting the amino acid L-arginine to L-citrulline and forming nitric oxide in the process.

While sceptics still thought it inconceivable that a biochemical pathway for producing nitric oxide was likely to exist in endothelial cells, they were batting against evolution. It transpires that the ability of organisms to produce nitric oxide is an ancient one, developed long before mammals emerged. Indeed, the horseshoe crab, with origins going back 500 million years, depends on the L-arginine: nitric oxide process to prevent its blood cells from aggregating.

The early research overturned some basic views of the cardiovascular system whereby blood pressure was seen as resulting from a constant balancing act between factors influencing the constriction and the dilation of vessels. Changes in blood pressure and blood flow happened if the balance was tipped too far one way or the other. Now, it seems, the normal cardiovascular state is tilted in one direction, and the dilation of vessels is sustained by a steady flow of nitric oxide. So any interruption to the production of nitric oxide interferes with the tone of the smooth muscle.

If normality means that blood vessels are dilated constantly, then the prevailing perception of blood pressure and the approach to managing hypertension may need a major overhaul. From the new point of view, raised blood pressure may be as much a problem of lack of dilator tone as it may be due to constriction caused by some unknown factor.

The new insight also explains at last the discovery made more than 100 years ago that a group of drugs, based on amylnitrite and nitroglycerine, could stop a painful attack of angina: the chest pain now known to be caused when the heart muscle is short of oxygen. By chance, the Victorian physicians had worked out that although it was too dangerous to give people nitric oxide directly, substances that released it slowly could relieve hypertension. The helpful effect on blood pressure of a whiff of amylnitrite vapor was recognized as long ago as 1867, and Conan Doyle credited Sherlock Holmes with that knowledge in The Case of the Resident Patient.

The poisonous effects of nitric oxide were well known. It was easy enough to make in the laboratory by adding copper turnings to concentrated nitric acid, and collecting the colorless gas over water. It nearly killed Sir Humphry Davy, in 1800, when he experimented with breathing it in.

In World War I, doctors noticed that workers packing shells with nitroglycerine in ammunition factories had very low blood pressures. The observation led to the development of a pill containing nitroglycerine that remains effective as an emergency vasodilator when popped under the tongue to give rapid relief for angina.

Although effective, the precise way in which the nitric oxide is released from nitroglycerine to imitate the L-arginine source of the molecule, is still a bit of a mystery. Nitric oxide is changed rapidly once in the bloodstream because it is highly attracted to the iron in hemoglobin.

As research into nitric oxide gathered momentum nine years ago, investigations spread way beyond the effect of the endothelial derived molecule. Three forms of the NOS enzyme were found: one in the endothelium, one in the brain and one in the immune system. Moreover, the biochemists were intrigued to discover that these enzymes were unusual in that they controlled a two stage reaction that would usually take two enzymes. More recently, molecular biologists have homed in on the different genes that encode for the endothelial, neuronal and immune system nitric oxide synthase enzymes: the genes are on chromosomes 12, 7 and 17 respectively.

From a clinical perspective, Patrick Vallance outlined the far-reaching implications of nitric oxide for treating various forms of cardiovascular diseases, which account for almost half the deaths in Britain. A vast range of research is aimed at translating the laboratory and animal findings about nitric oxide for the benefit of the cardiovascular and heart disease sufferers.

His group used healthy volunteers to test whether nitric oxide acted on the human cardiovascular system in an identical way to that predicted from in vitro and animal work in the laboratory. The study entailed what has become a new class of drug based on N-monomethyl-arginine (L-NMMA - an inhibitor of the NOS enzyme). This drug is one of a class of inhibitors that can be used to explore what happens when nitric oxide production is blocked. In the experiment, one forearm of a volunteer was injected with L-NMMA. The blood flow was then compared with that of the other arm. As L-NMMA was infused gradually, blood flow decreased to a half of that in the control arm.

Complications caused by problems with endothelial production of nitric oxide arise from a variety of causes: the fragile layer of endothelial cells are prone to damage, from among other things, high blood pressure, high sugar in diabetes, furring up with cholesterol and other lipids, and the effect of smoking.

In cases involving a risk of heart attack, attention focuses on nitric oxide deficiency, and the dangers when its release is blocked from a diseased coronary artery so that vasodilator tone needed to regulate the flow of blood is lost. Other diseases can also be treated by increasing the supply of nitric oxide to blood vessels. A trace of nitric oxide gas (25 parts per million) inhaled by patients with pulmonary hypertension can relieve lung congestion. In a treatment for newborn babies, breathing problems are being helped by inhalation of nitric oxide that relaxes constricted blood vessels and dilates the lung's blood vessels.

Generally, low blood pressure is not a problem in healthy people. Occasionally, it is a symptom of serious condition; and an acute drop can signal that an overwhelming infection has caused septic shock and severe tissue damage. The trouble lies in a vicious cycle. Septic shock depresses blood pressure. The loss of blood flow can cause tissue damage, and that in turn increases the production of nitric oxide, leading to a further fall in blood pressure. Low blood pressure induced by septic shock has been restored with a treatment that included the use of an NOS inhibitor.

The remarkable role nitric oxide plays as a messenger between nerve cells was revealed by John Garthwaite and colleagues, at Liverpool University, who first identified it in the brain. Subsequent measurements have shown that the brain contained more of the nitric oxide synthase enzyme than any other organ.

When research into the possible role of nitric oxide in the central nervous system began, the brain was the least expected organ in which to find the molecule. It transpired that the nitric oxide synthase enzyme was not confined to small areas, but was distributed throughout the brain: suggesting an involvement in almost every brain function. It turns out that because the molecule is so small, it is a physically convenient messenger that diffuses into and out of cells easily; and it is now a prime candidate for the much searched for "retrograde messenger" that is the basis of memory.

Animal studies suggest that an effector cell in the brain which releases the chemical messenger glutamate can stimulate the receptor cell with this chemical to release nitric oxide. If it is strongly stimulated, the receptor sends back a nitric oxide molecule to tell the sender that the message has been received, and programs it to send an even stronger signal next time - the phenomenon of long term potentiation that is thought to be linked to memory formation.

Nitric oxide produced in the brain by the neuronal form of the nitric oxide synthase acts as a chemical messenger at the synapses. The in-vitro and animal experiments showing the role of neuronal nitric oxide in memory has opened a new approach to studies of Alzheimers, Parkinson's and other neurological disorders.

Too much nitric oxide, on the other hand, may be responsible for killing brain cells in conditions such as stroke.

In yet another role, nitric oxide helps the immune system's macrophages, the cells in the body that are activated in injury and illness to get rid of debris, when they seek out invading bacteria or cancer cells to destroy. As the "bin men" of the immune system, the macrophages use the cytotoxic properties of nitric oxide to sweep up parasites, bacteria and other potentially infectious rubbish.

Eddy Liew's work on the role of nitric oxide in the immune system shows the molecule will destroy many organisms that are difficult to kill by other means. And his group has demonstrated the susceptibility to nitric oxide of the vicious parasite that causes Leishmaniasis, a group of potentially fatal tropical diseases caused by parasites transmitted by the bite of sandflies.

Treatment with the inhibitor L-NMMA also appears to block inflammation in the body associated with the development of arthritis and kidney disease. Moreover, mice genetically engineered not to generate nitric oxide during the immune responses display a reduced inflammatory response. If research now underway confirms the scientists' hypotheses, the advances could lead rapidly to a new class of drugs to control inflammatory diseases that are classified as auto-immune diseases because the affected tissue is under continual attack by its own immune system.

Research on nitric oxide, its physiological and pathological roles and clinical potential is currently one of the most exciting sources of knowledge in biology.

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INFORMATION NOTE

Nitric oxide: from menace to marvel of the decade

On 31 May 1995, the Royal Society and Association of British Science Writers held a scientific press briefing on Nitric oxide: from menace to marvel of the decade. The enclosed document was prepared afterwards to summarize key issues raised by the speakers and to provide a list of helpful contacts for future reference.