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# Nitric oxide: a challenge to chiropractic

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*The 1998 Nobel Prize in Physiology or Medicine recognized the biological significance of nitric oxide. Nitric oxide is derived from the amino acid arginine. It is intimately involved with circulatory vessel dilation where, for example, it protects against heart attacks, and is the basis for new medications such as Sildenafil (Viagra). Nitric oxide acts as a neurotransmitter and can modulate many neurological reactions. The immune system uses nitric oxide to destroy pathogens by interfering with key enzymes. Nitric oxide is responsible for both osteoclastic and osteoblastic responses in bone and is a key player in the degenerative aspects of arthritis. The process of apoptosis employs nitric oxide in the orderly removal of unneeded cells. There is clear evidence that major signaling and control mechanisms exist in the body apart from the nervous system. Chiropractic is thus faced with the challenge of how to incorporate this new knowledge which conflicts with traditional chiropractic concepts.*
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*Le lauréat du Prix Nobel 1998 de physiologie ou de médecine a reconnu l'importance biologique de l'oxyde nitrique, un dérivé d'un acide aminé, l'arginine. L'oxyde nitrique intervient dans la dilatation des vaisseaux; par exemple, il protège le cœur contre les infarctus et il est à la base de nouveaux médicaments comme le sildénafil (Viagra). Il agit également comme neurotransmetteur et peut modifier de nombreuses réactions neurologiques. Par ailleurs, le système immunitaire utilise l'oxyde nitrique pour détruire des agents pathogènes en agissant sur des enzymes clés. Il est aussi responsable des réactions ostéoclastiques et ostéoblastiques et joue un rôle important dans la dégénérescence des tissus observée dans l'arthrite. En outre, l'oxyde nitrique sert dans l'apoptose pour la destruction programmée des cellules inutiles. Voilà une preuve évidente qu'il existe, à part le système nerveux, des mécanismes importants de transmission de signaux et de commande dans l'organisme. La chiropratique fait face maintenant à un dilemme : comment intégrer ces nouvelles connaissances qui entrent en contradiction avec les concepts traditionnels de la chiropratique?*
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MOTS CLÉS : oxyde nitrique, chiropratique.

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## Introduction

Numerous chemical bio-messaging and signaling mechanisms have been found necessary for human function. While beyond the scope of this paper these mechanisms include integrin transmembrane signaling,[1] sequential protein kinase reactions,[2] and antigen receptor signaling.[3]

This paper is intended as an overview of the remarkable bio-messaging role played by nitric oxide (NO). A medline search found more than 18,000 peer-reviewed, indexed papers have been published on the topic of nitric oxide in the past four years. Thus, a comprehensive review is not possible in this paper. Instead, those references were chosen that provided a summary of current nitric oxide research, and which held potential implications for chiropractic practice and philosophy.

An October 12, 1998 press release announced the Nobel Prize in Physiology or Medicine for 1998 was awarded to Robert F. Furchgott, Louis J. Ignarro, and Ferid Murrad for their discoveries concerning "nitric oxide as a signalling molecule in the cardiovascular system."[4]

In 1980 Furchgott revealed that the vasodilative action of acetylcholine no longer worked if the inner endothelial lining of blood vessels was removed. The presence in the lining of an endothelium-derived relaxing factor (EDRF) was needed for vaso-dilation to occur. EDRF was necessary for the formation of cyclic guanosine monophosphate (GMP), a messenger for neurotransmitters and hormones.[5] Further research identified EDRF as being identical with the gas nitric oxide (NO).[6]

Recognizing its future potential the journal *Science* named nitric oxide 'Molecule of the Year' in 1992.[5]

## From humble origins

NO was first discovered in 1772 by Joseph Priestly who also discovered oxygen. NO, a colorless, highly reactive gas, normally exists for only a few seconds before combining with oxygen to form nitrates. Possessing an extra electron, it is a highly reactive free radical in the body. Its toxic-like behavior initially gave no clues that it might play a crucial role as a mediator of body function.[7]

It was initially observed that subjects fed low-nitrate diets still excreted considerable amounts of nitrates. Rats subjected to bacterial endotoxin injections also showed increased nitrate excretion. Further experimentation revealed an association between the level of macrophage activity and the presence of nitrates.[5]

Pursuing the macrophage issue further it was discovered that macrophage defense required the amino acid arginine to produce nitrates. Macrophages use an intermediary enzyme to convert the arginine into nitric oxide and citruline. The nitric oxide resulting from the conversion of arginine permits macrophages to kill foreign invaders by its toxic, free radical activity.[5]

## Biochemical synthesis of NO

Nitric oxide is formed by oxidation of the amino acid L-arginine. The catalyst nitric oxide synthase (NOS) drives this process. NOS may be either constitutive (constant production) or inducible (iNOS) in form. Each form reflects differing origins, chemical makeup, and function. Constitutive NOS I is found in nerve cells and is involved in nervous system signal transduction. Constitutive NOS III is found in endothelial cells and regulates blood flow and platelet function. Constitutive NOS levels are regulated by activating or inhibiting enzymes.[8,9]

The constitutive forms of NOS are a key regulator of homeostasis, whereas iNOS is involved in inflammation, defense, and repair.[10]

iNOS is expressed as needed by nearly all body cells, particularly white blood cells and hepatocytes. Cytokines such as tumour necrosis factor alpha (TNF–$\alpha$), interferon gamma (IFN–$\lambda$), and certain interleukins (esp. IL–1, IL–2) are the primary inducers of iNOS. [8]

## Physiological effects of NO

Nitric oxide's short half-life of 5–10 seconds limits its actions to the immediate vicinity. After entering a cell, nitric oxide commonly targets the enzyme *guanylate cyclase*. This in turn catalyzes the formation of cyclic GMP. Cyclic GMP then activates a intracellular signal to evoke the cell's ultimate response.[11] Other molecules targeted by NO include haem proteins and superoxide anions in vascular and cardiac tissue.[12,13] NO further reacts with oxygen free radicals to produce peroxynitrite, a powerful oxidant.[14]

The physiologic effects of NO are highly dose dependent, with toxic effects generally increasing with higher doses.[13,15] The ultimate effects of NO are dependent not only upon dosage levels, but also on the rate and timing of NO release, and on the level of reactive oxygen concentration.[14]

**NO as a neurotransmitter and blocker**

It is counterintuitive to consider a toxic, highly reactive gas such as nitric oxide as a crucial neurotransmitter. Neurotransmitters are normally stored as stable chemicals in synaptic vesicles of nerve terminals. Upon release they fuse with receptor proteins on adjacent neurons and commence signaling cascades. In contrast, nitric oxide does not rely on vesicle storage. NO is synthesized as needed whereupon it promptly diffuses in all directions into nearby neurons, bypassing conventional neural receptors.[5] The signaling action of NO is both intra- and intercellular.[16]

Nitric oxide may exhibit either a up- or downregulatory mode in the spinal cord depending upon the types of fibers activated and the intensity of signal input.[17]

In addition to its role in neurotransmission NO is also involved in neural development, neural regeneration, and regulation of genetic expression. Nitric oxide is further considered to mediate both nociceptive and proprioceptive transmission.[18,19]

In the gastrointestinal tract NO is likely a transmitter for inhibitory motor neurons, but not in enteric sensory neurons. NO modulates intestinal reflexes by inhibiting transmission in the descending reflex pathway. The site of activity appears to be between sensory neurons and interneurons. In this location NO acts as a retrograde transmitter inhibiting transmission of sensory neurons.[20]

Observations indicate that NO modulates intestinal reflexes by direct action on intestinal neurons of the enteric nervous system. NO released by interneurons act as negative feedback system on sensory synapses inhibiting transmission.[20]

Additional research has established that electrical vagal stimulation produces stomach relaxation through NO release.[21] NOS expression, and thus NO release, in the rat gastric myenteric plexus is under vagal control.[22]

In a rat model of diabetes gastric relaxation is impaired. The impaired relaxation results from diabetes related reduction in NOS-immunoreactive cells with corresponding loss of NOS expression and decreased gastric relaxation.[23]

NO may also serve as a neurological amplifier by strengthening a postulated feedback loop between postsynaptic and presynaptic cells. NO can serve as an amplifier of calcium signals in neuronal cells. In the presence of NO weak input signals that might otherwise be unnoticed by the cell can undergo amplification and result in significant physiological responses.[24]

NO mediated inflammation causes neural conduction block in multiple sclerosis and Guillain-Barré syndrome.[25] Exposed to inflammatory levels of NO peripheral nerves can experience a total loss of compound action potential in both myelinated and unmyelinated fibers.[26]

While excessive levels of NO cause neural injury, normal levels play a key role in neural development, regeneration, and regulation of gene expression.[18] Nitric oxide's ability to modulate synaptic gap junctions is a probable mechanism whereby cortical modulation and subcortical pattern formation occurs.[27]

**The vascular connection**

It is recognized that reduced nitric oxide is a factor in the development of cardiovascular disease. This may occur from abnormal signal transduction, insufficient L-arginine, and lowered enzyme expression.[28,29] It has also long been recognized that cardiovascular dysfunction often results in male impotence due to penile vascular perfusion deficits.[30] The presence of cardiovascular disease interferes with the prerequisite smooth muscle relaxation.[31] This realization led to investigations of a possible role for NO in erectile dysfunction.

It was soon discovered that release of NO from either the vascular endothelium or from non-cholinergic, non-adrenergic nerves would increase intracellular levels of cGMP (cyclic guanosine monophosphate). Increased levels of cGMP in turn modulated intracellular calcium promoting erectile function.[32]

Certain phosphodiesterase compounds appeared to play a crucial down regulatory role in the levels of cGMP.[33] Thus a search for compounds to inhibit the action of phosphodiesterase, thus increasing levels of cGMP, led to the discovery of Sildenafil citrate (Viagra).[34] Sildenafil inhibits phosphodiesterase allowing increased cGMP thereby releasing intracellular calcium. The calcium binds to calmodulin activating nitric oxide synthase (NOS). NOS releases nitric oxide producing smooth muscle relaxation and enhanced erectile function.[5,33]

This research also explains why nitroglycerin, the active ingredient in dynamite, is effective as a treatment for certain heart problems. Nitroglycerine is metabolically converted to nitric oxide which dilates the coronary arteries.[5]

**Immune system modulation**

The immune system depends upon the ability of thymus-derived (T) lymphocytes to recognize foreign antigens. T cells are further divided into helper T cells (Th) and cytotoxic (Tc) cells. Upon activation, Th cells produce lymphokines that regulate other cells of the immune system. The types of lymphokines produced further categorize Th cells. Th1 cells produce IL-2 and gamma interferon while Th2 cells produce IL-4 and IL-5.[35]

It has been demonstrated that cytokines from T helper 1 cells (Th1) increases NO production. Conversely, T helper 2 cell (Th2) derived cytokines inhibit NO production suggesting immune helper cells regulate NO production.[36]

Levels of NO must be carefully regulated within narrow parameters. Abnormal levels of NO are closely associated with numerous autoimmune pathologies. These include rheumatoid arthritis, surgical graft rejection, diabetes, lupus, and multiple sclerosis. NO levels are regulated, in part, by opposing cytokines from Th1 and Th2 cells. NO may in turn prevent excess numbers of Th1 cells that are implicated in severe immunopathology.[37]

A variety of studies have demonstrated the NO role in antimicrobial defense against such intracellular pathogens as *Leishmania, Toxoplasma, Trypanosoma, Listeria,* and *Rickettsia* in rodents.[38] In humans the NO produced by neutrophils, macrophages, and other cells inactivate bacterial enzymes.[8]

**Apoptosis**

Living cells eventually die from either necrosis or apoptosis. In necrotic death cells passively swell, mitochondria are disrupted, the cell membrane is lysed, and cellular contents are released into surrounding tissues causing local edema and swelling.

The other form of cell death, apoptosis (Gr. "a falling off"), is the process of controlled cell suicide whereby cells no longer needed by the body are eliminated. In apoptotic death an active process of cell shrinkage occurs, followed by phagocytosis preventing nearby tissue inflammation or damage.[39]

Controlled apoptosis is crucial to normal health. The process of embryonic growth and differentiation requires that surplus cells die and be removed. In later adult life, disrupted apoptosis allows development of neoplastic cells and certain autoimmune diseases. Excessive apoptosis, however, is likely involved in neurodegenerative diseases and in diabetes.[39]

NO has been termed the 'mediator of lethal processes' from its close association with apoptosis. One common mechanism involves NO reaction with superoxide anions. When the NO concentration reaches critical levels, determined by cell type and the local environment, regulated cell death occurs.[39]

NO mediated apoptosis is implicated in many neurodegenerative diseases, including Alzheimer's, Parkinson's, and cell death in cerebral ischemia.[39,40] Combining with certain cytokines NO is a primary mediator of apoptotic cell death in osteoblasts.[15]

NO at sufficient levels depresses myocardial contractility and is toxic to cardiac myocytes.[14] NO induces apoptosis in cortical neuronal cells.[41] By causing enterocyte apoptosis NO produces "bare areas" in the intestinal epithelium which are then susceptible to bacterial invasion and a subsequent systemic inflammatory response.[42]

Conversely, NO demonstrates a protective effect against actinomycin induced liver apoptosis in mice.[43] Thus, depending upon the local tissue environment, NO may act as either a pro- or anti-apoptotic molecule.[44]

**Inflammation and arthritis**

In the body NO lasts only a few seconds before being oxidized to nitrite and ultimately excreted as nitrate. Monitoring urinary nitrate gives a measure of NO activity. Low amounts of NO in the body are protective against inflammation damage by maintaining tissue perfusion. As will be demonstrated later, increased levels of NO are destructive, giving NO a 'double-edged sword' reputation.[8]

Cerinic investigated the potential role of the nervous system in arthritis and determined that inflammation can modulate efferent and afferent portions of the peripheral nervous system (PNS).[45] Further, afferent (nociceptive) terminal fibers may have an efferent effect by releasing local neuropeptides and proinflammatory mediators.[45]

NO not only reveals, but also mediates the progression of arthritis. Numerous studies have confirmed that NO is a marker for, and a pro-inflammatory mediator of, arthritis. Adjuvant induced arthritis results in nitrate excretion, reflecting the action of NO that corresponds with arthritis progression. A similar pattern exists for systemic inflammatory joint disease. Increased nitrite levels are found in the joints of patients with rheumatoid arthritis (RA), osteoarthritis (OA), and in the spondylarthropathies. NO

further appears to be involved in the pathogenesis of systemic lupus erythematosus (SLE) and chronic inflammatory bowel diseases.[8]

A variety of cells are sources of NO in arthritis, including macrophages, synoviocytes, endothelial cells and chondrocytes. Not only is this NO a likely factor in arthritis and related inflammation, it appears to also help develop and maintain the associated hyperalgesia. NO thus joins substance P and other neuropeptides in the potentiation of pain.[46,47]

It has been demonstrated that articular symptoms and joint degeneration in animal models can be reduced by use of NOS inhibitors to block NO release.[10]

The actions of many arthritis drugs are directed at NO activity. Prednisolone reduces joint nitrite levels, salicylates act to scavenge NO, cyclosporin drugs inhibit NOS expression, and methotrexate blocks cofactors of NOS.[8]

In human osteoarthritis the synovial fluid contains few neutrophils or leukocytes, common sources of NO. Instead osteoarthritis involves a change in the balance of catabolic and anabolic activities in the collagen matrix. It is believed that articular chondrocytes have the capacity to express sufficient NOS to cause cartilage degeneration and is likely a factor in the pathogenesis of osteoarthritis.[10]

In osteoarthritis (OA) fundamental alterations occur in the cartilage tissue itself, different from that found in rheumatoid arthritis (RA). In OA inflammation mediators are found in the chondrocytes themselves. Chondrocyte staining of cartilage from patients with OA or traumatic arthritis revealed elevated levels of the cytokines interleukin (IL–1ß) and tumor necrosis factor (TNFα) and marked elevations of iNOS, considerably more than in the inflammatory arthritides. The coordinated autocrine involvement of these three substances appears to be involved in the pathogenesis of many arthropathies.[48]

It is presumed that trauma and/or repeated microtraumas provide the trigger to initiate degenerative NO and cytokine activity.[48] One study suggested a role for collagenase production in the development of osteoarthritis.[49]

In rheumatoid arthritis (RA) expression of iNOS is highest in the joint synovium, the synovial lining, blood vessels, and somewhat in the cartilage. This is in contrast to OA where no iNOS expression occurs in the synovium. The early feature of RA is local bone loss. This results from the inhibitory action of NO on osteoblast formation. NO likely plays a key role in the joint swelling and vasodilation characteristic of RA. When inhibitors of NOS are used they suppress the development of inflammatory joint disease.[50,51]

The data suggest that certain synoviocytes, the cells of the synovial membrane, are derived from monocytes. These synoviocytes, known as $CD14^+$, express iNOS and produce NO. The number of $CD14^+$ cells present correlates closely with subsequent production of NO.[52] The NO released by the synoviocytes accumulates at a point of injury activating nociceptors directly and causing vasodilation, inflammation, and swelling.[53]

Further investigation into the pathogenesis of inflammatory arthritis suggests NO, in concert with certain cytokines, suppresses proteoglycan signaling and induces chondrocyte apoptosis.[54,55]

**Bone remodeling**

Bone resorption by osteoclasts is a complex process involving cytokines, hormones, and other components. Osteoblastic production of NO serves as an intercellular inhibitor of osteoclastic action. Conversely, osteoclasts can produce NO in response to inflammation. Osteoclastic NO serves to dampen excessive bone resorption.[56]

The effects of NO on bone remodeling are extremely dose sensitive. Osteoblasts can release NO which in turn regulates osteoblast growth. When levels of NO pass a certain threshold, as in inflammatory conditions, new bone formation is reduced. Beyond this threshold NO becomes inhibitory to osteoblasts and possibly toxic. Excess levels of NO are likely involved in other bone depleting diseases including osteopenia, postmenopausal osteoporosis, tumor-associated osteolysis, and periodontal disease.[57,58] NO thresholds for osteoclast and osteoblast activity may not be identical, however. Thus a given level of NO may upregulate or downregulate either osteoblasts or osteoclasts, or both, depending on local conditions.[55,58]

Estrogen has been shown to protect against cardiovascular disease and osteoporotic bone loss in postmenopausal women. The benefits from estrogen therapy derive from estrogenic stimulation of osteoclasts to generate NO, which in turn upregulates osteoclast activity.[57,59]

Disruption of normal bone homeostasis by excessive NO results in not only joint deterioration but also in loosening of joint prostheses. As polyethylene components of the prostheses flake off macrophages are attracted to these

debris particles. Phagocytosis of these particles is followed by expression of iNOS and subsequent release of NO. The resulting bone resorption around the implant causes aseptic loosening and eventual failure of the hip prosthesis.[60,61]

**The multifunctional molecule**

Nitric oxide's role as a multifaceted biological mediator is pervasive. From near obscurity fifteen years ago nitric oxide is now considered one of the major players in physiological function. Nitric oxide is involved in the dilation of blood vessels, helps defend against infection and tumors, serves as a neurotransmitter in the nervous system, and as a contributor to remodeling of bone. It is an essential agent of apoptosis, and is involved with numerous cytokines in the regeneration and degeneration of many body tissues. Nitric oxide mediates biochemical cascades of events that serve to control or effect any of a number of bodily functions. It effects this control in response to local biochemical signals and processes.

Many biological responses are initiated in response to local events. Severing of blood vessels starts a biochemical cascade of events leading to the clotting of blood. This biochemical cascade is subject to control by nitric oxide. NO can inhibit platelet aggregation[62] and can reduce the clotting action of factor XIII.[63] Hemoglobin itself is being increasingly seen as a carrier and distributor of nitric oxide along with oxygen.[13]

The immune response is triggered when an antigen enters the body and is chemically recognized as being foreign. Activated lymphocytes converge and eliminate the invader by producing NO which deactivates key enzymes in the target pathogen.[38]

It is known that anxiety disorders can affect the immune system by reducing lymphocyte cytokine and NO production.[64] Likewise, NO can modulate the firing of neural synapses, influence the proliferation of nerve terminals, produce retrograde synaptic firing from afferent fiber terminals in an efferent manner, and support nociceptive transmission.[20,24,47]

Nitric oxide's role is so pervasive that it even plays a key role in disease resistance in plants by inducing genes to synthesize protective enzymes.[65]

**Implications for chiropractic**

Chiropractic's classical position regarding health and the regulation of body functions has been a simplistic and straightforward one. D. D. Palmer expressed the basic concept as:

> *"The cause of disease is a mechanical obstruction to natural functions."*[66]

Palmer's hypothesis of nerve interference causing disease reflects his understanding of the limited scientific knowledge of his time. However, Palmer's concept of nervous system blockage as the basic cause of disease still finds expression today:

> *"Because the nerves work as a conduit of the life force, interference to the normal quantity flow causes malfunction. This is the fundamental basis of dis-ease."*[67]

Dramatic advances in scientific understanding of human physiology bring these classical chiropractic explanations of human function into question. Developing knowledge of human biological messaging systems, as noted in this review of the role of nitric oxide, lends little or no support to traditional chiropractic concepts. Quadriplegics, with massive 'nerve interference', continue to demonstrate normal immune system function in the complete absence of neurological input.

As illustrated previously, many regulatory functions of the body take place with limited or no input from the nervous system. Indeed, nitric oxide is itself a potent modulator of the nervous system. Local conditions in the body have the capacity to exert a controlling influence back on the nervous system via nitric oxide and cytokine intermediaries.

This should not be seen as minimizing the importance of the nervous system. Rather it is an attempt to gain a rational perspective that recognizes body systems work as a coordinated whole. One example of this broadened perspective notes: "The skin, the nervous system and immunity are not independent systems but are closely associated and use the same language of cytokines and neurotransmitters."[68] Another researcher notes that the immune system is itself a sensory organ, that bi-directional communication pathways exist with the nervous system, and that the immune system influences other elements of physiology.[69]

This broader and more egalitarian view sees physiologi-

cal function as a seamless interlocking process with no one system necessarily superior. It is increasingly clear that chiropractic notions about human health and function being dependent upon removing 'nerve interference' are inadequate, misleading and hopelessly simplistic.

The challenge to chiropractic is to adapt its perspectives to the latest in scientific understanding. The chiropractic profession could choose to continue limiting itself to the dictates of its archaic and arbitrary tradition. The other choice is to deal with the implications of new knowledge about the body's sophisticated systems of signal transduction and control. These bio-chemical signaling systems are clearly at odds with traditional chiropractic theory.

To create an inclusive model of care chiropractic must expand its views to include the body's complex signaling systems. Such a move would allow better definition of those conditions most amenable to our therapeutic interventions. For chiropractic to lay claim to being a bona fide health science we must incorporate the latest in scientific knowledge, even when it means abandoning old traditions.

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