

Nitric oxide and its role in health and diabetes.

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Summary Overview

Nitric Oxide

Nitric oxide (NO), is a free radical gas that is a powerful regulator of circulation (it is an endogenous vasodilator) and a neurotransmitter (it helps in the processing of nerve signals as they cross synapses). L-arginine, one of 20 amino acids that make up proteins, is the only amino acid that generates significant amounts of NO.

The enzymes that produce NO from L-arginine

Nitric Oxide Synthase (NOS) is the enzyme that generates NO from L-arginine. This enzyme exists in three different forms (called isoforms), NOS 1, NOS 2 and NOS 3. Each isoform synthesizes NO but does so under different conditions. Often all three isoforms will be found in the same cell but occasionally one cell will contain only one of the isoforms. This is important because many see or hear the term **nitric oxide** and assume that it refers to all cells under all conditions. Each of the three isoforms is described below.

1. NOS1 is the neural (or brain) isoform, sometimes referred to as bNOS. It helps in synaptic transmission, the processing of nervous information from nerve to nerve across gaps between the nerves called synapses and from peripheral nerves to the brain.
2. NOS2 is called inducible or iNOS. This enzyme generates extraordinarily high concentrations of NO, in part to kill bacteria. NOS2 (iNOS) takes several hours to be mobilized and the response is due to an injury or infectious process. NOS2 produced by macrophages is responsible, in part, for their effects to repair injury and to ward off infections. Extraordinarily high concentrations of NO (100 to 1000 times normal) are produced very locally by this isoform. Unlike NOS1, which is part of normal neurotransmission, there must be something very abnormal (a wound, tissue damage, hypoxia, bacterial infection, etc.) to induce this enzyme.
3. The third isoform is eNOS (or NOS3) which stands for "endothelial cell" NOS. This isoform is active at all times (it doesn't need to be induced as does iNOS) and is found in endothelial cells which are the cells that line the inner surface of all blood vessels and lymph ducts. EcNOS is activated by the pulsatile flow of blood through vessels (the stretching and relaxation of the blood vessel wall in response to each beat of the heart). This leads to a "shear stress" on the membrane of the endothelial cells as the column of blood in the vessel moves forward and then stops. This NO,

produced by eNOS, maintains the diameter of blood vessel so that perfusion of tissues (skin, muscle, nerves, and bone) is maintained at optimal levels. In addition, eNOS mediated NO causes angiogenesis, which is the growth of new blood vessels. This is especially important in healing an ulcer or wound on the skin.

NO initiates and maintains Vasodilation

NO initiates and maintains vasodilation through a cascade of biological events that culminate in the relaxation of smooth muscle cells that line arteries, veins, lymphatics. While somewhat complex, the sequence of biological events that are triggered by NO is described below:

Step 1. NO gas released from nitrosothiols in hemoglobin or from endothelial cells diffuses into smooth muscle cells that line small blood vessels.

Step 2. Once inside the smooth muscle cell, NO binds to an enzyme, called guanylate cyclase (GC) and this binding results in GC activation.

Step 3. Activated GC is able to cleave two phosphate groups from another compound called guanosine triphosphate (GTP). This results in the formation of cyclic guanosine monophosphate (cGMP) that is used to phosphorylate (Phosphorylation is the addition of a phosphate group) proteins, including the smooth muscle contractile protein called myosin.

Step 4. Once phosphorylated, smooth muscle cell myosin relaxes, resulting in dilation of the vessel that was originally exposed to NO.

This vasodilation continues until a phosphatase enzyme dissociates the phosphate from myosin (which may be delayed by Viagra). Since vasodilation through NO only occurs when there is GC able to bind NO, additional NO, is sequestered for future use as a nitrosothiol, including those found in hemoglobin.

NO influences neurotransmission and mediates pain

NO has both a direct and indirect influence on neurotransmission. NO, by affecting cGMP, allows phosphorylation of ion channels, especially potassium channels necessary for normal transmission of nerve signals. NO also increases blood flow. This allows sufficient oxygen and glucose to be transported to nerve cells, positively affecting ATP production and, in turn, potassium/sodium homeostasis essential for neurotransmission. Increases in blood flow may also allow the oxygen dependent isoform, bNOS, to produce more NO.

In addition to improving neurotransmission, NO functions to reduce pain. No reduces pain directly by increasing cGMP (the mechanism by which opioids work), and indirectly by increasing circulation to restore normal membrane potential and reduce pressure on nerves due to localized edema.

NO is important in the process wound healing and tissue repair

Nitric Oxide (NO) and its interrelationship with essential growth factors is critically involved in the entire continuum of events associated with wound repair. NO is a powerful stimulator of cell division (proliferation) and maturation, particularly formation of appropriate cell receptors (differentiation). NO is a necessary mediator of neovascularization, i.e., the formation of new and eventually mature blood vessels (angiogenesis) and lymph ducts to nourish the healing tissues. NO increases the number of fibroblasts (fibroblastic proliferation) and thereby enhances collagen formation for the healing wound. Lastly, L-arginine and NO are necessary for the proper cross-linking of collagen fibers to one another, via proline, to minimize scarring and maximize the tensile strength of healed tissue.

NO is often impaired in people with diabetes

Both Type I and Type II diabetic patients have a reduced ability to generate NO from L-arginine, reflected in part by direct measurements of plasma nitrate and nitrite levels. Several factors influence nitric oxide production and metabolism.

As part of normal metabolism of L-arginine small amounts of a natural inhibitor of NOS are formed (asymmetrical dimethyl arginine (ADMA)). Normally, ADMA does not accumulate in the blood because it is rapidly eliminated in the urine through normal kidney function. Reduced kidney function as part of aging (more than 20% of all Americans over 65 have Type 2 diabetes) or due to kidney dysfunction, which is accelerated by diabetes, may prevent the elimination of the major NOS inhibitor, ADMA, thereby limiting the production of NO.

Nitric oxide synthase (NOS) from which NO is derived, is a pH dependent enzyme. It is active at slightly alkaline (basic) conditions but is suppressed by acidotic conditions. In diabetes, glycolysis and ketoacidosis force pH toward acid conditions and this may account, in part, for the reduced production of NO.

Adequate Oxygen is necessary for the activity of NOS and therefore NO. Circulation is notoriously impaired in diabetic patients, which limits available NOS and NO.

Lastly, people with diabetes often experience elevated glucose levels. Some of this glucose becomes incorporated into hemoglobin and is measured as glycosylated hemoglobin (Hgb) or HgbA1C. Glycosylated hemoglobin binds NO in the form of nitrosothiols very tightly so that any NO that is formed cannot be easily released from RBC to help maintain blood flow through smooth muscle cell relaxation.

In summary, acidosis, low oxygen, and/or accumulation of ADMA are responsible for the decreased production of NO. What NO is available is tightly bound to glycosylated hemoglobin limiting its release and smooth muscle cells where NO affects essential cellular functions.

NO deficiency may impair the health of people with diabetes

Reduced production and higher than normal binding, may be partly responsible for the poor circulation in diabetic patients and would be one of the reasons for their high propensity to develop an ulcer. Additionally, poor nitric oxide metabolism is thought by some researchers to be the cause of peripheral neuropathy, the nerve damage often observed in people with diabetes.

In understanding the ways that NO can reduce pain, it is easy to realize its significance in people with diabetes. Impaired circulation is a typical consequence of this disease. Disturbed membrane potential would be anticipated thus decreasing the stimuli necessary for nerve firing and perception of pain. Additionally, this impaired circulation often leads to swelling in the extremities, exerting pressure on the nerves, which also causes pain. Lastly, NO mediated increases in cGMP may be impaired limiting its ability to directly reduce neuropathic pain.

Dr. Tom Burke received his PhD in Physiology from University of Houston, Post Doctoral Training at Duke Medical School, He was an Associate Professor of Medicine and Physiology at the University of Colorado Medical School. He has authored more than 70 published scientific clinical articles and has been a visiting scientist at the Mayo Clinic, Yale University, University of Alabama, and University of Florida. He is a recognized international lecturer on cell injury and nephrology.