

Nitric oxide and its role in health and diabetes.

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Part 2

Isoforms of Nitric Oxide Synthase

Nitric Oxide Synthase (NOS) is the enzyme that generates NO from L-arginine as described in Part 1 of this series. However, the enzyme exists in three different forms called isoforms. 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. This is not the case as outlined below.

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.

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. In other words, when the body mounts an inflammatory response to injury, macrophages are attracted to the site of injury where they produce large amounts of NO. Extraordinarily high concentrations of NO (100 to 1000 times normal) are produced very locally by this isoform. In fact, reports suggest that wound (ulcer) fluid may contain levels of NO that are very high and can only be attributed to iNOS. 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. For the wound community that event is usually anything that threatens integrity of the skin.

The third isoform is ecNOS (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. What does pulsatile mean? It is the stretching and relaxation of the blood vessel wall in response to each beat of the heart. Each time the heart beats it leads to an acute increase in the diameter of the blood vessel, followed by an equally acute return to a normal diameter. 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

ecNOS, maintains the diameter of blood vessel so that perfusion of tissues (skin, muscle, nerves, and bone) is maintained at optimal levels. In addition, ecNOS 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.

One interesting interplay of iNOS and ecNOS is in tissue repair. Initially, NO is generated from iNOS in order to ward off infection and to destroy and remove the irreversibly damaged, necrotic tissue. This is often referred to as the inflammatory stage of wound repair. This phase lasts only a short time (a few days with an acute wound) and then ecNOS is (or should be) mobilized to cause vasodilation and angiogenesis to induce the healing response. NO will relax smooth muscle cells and thus dilate veins, arteries, and lymphatics. This increases blood supply both to the repairing tissues and from the damaged region. The latter removes metabolic waste products, reduces edema, and prevents swelling that would otherwise compress capillaries. In the absence of adequate blood supply tissue will remain hypoxic and heal only slowly, if at all. Moreover, since iNOS is produced in large part by white blood cells (WBC), vasodilation permits delivery of additional WBC to the area that needs to be defended from infection. There are wounds that do become infected and often only marginal reduction of the infection is seen even with high dose and high potency antibiotics. By now most should realize that if the vascular bed (arteries, veins, and lymphatics) were dilated, more of the antibiotic would get to site of infection. Thus it is essential that ecNOS be activated to produce NO. Clearly both ecNOS and iNOS play a role in wound healing; neither alone is sufficient to achieve full recovery. In diabetic patients, however, ecNOS activity is often well below normal so these patients cannot produce NO at normal levels.

Finally, NO generated at physiologic levels, via ecNOS, will suppress the activity of the enzyme iNOS. This is why there is usually only a transient increase in iNOS activity in the normal response to wounds or tissue damage. In diabetic patients, with low production of NO from ecNOS, iNOS may not be inhibited and iNOS mediated NO production may remain high well beyond its intended time. This could contribute to continuous and uncontrolled tissue destruction, thereby slowing the healing process.

We have not fully explored bNOS or brain (neural) NOS activity in this discussion. However, as we develop the theme of reversal of diabetic peripheral neuropathy later in this series of articles, one should remember that all three isoforms of NOS including bNOS, require molecular oxygen in order to function appropriately. Clearly, neural transmission (sensation of pain, pressure, and temperature) will be impaired if circulation (delivery of oxygen) is impaired. Thus, synaptic transmission and the proper processing of nervous stimuli need oxygen (controlled in part by ecNOS) in order for bNOS to carry out its NO mediated activity.

In the next part of this series we will discuss how NO formation and/or availability, especially from ecNOS, is altered in the diabetic patient.

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