PATHOPHYSIOLOGY OF SEPSIS: THE ROLE OF NITRIC OXIDE

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In 1998, the Nobel Prize for Physiology and Medicine was awarded to Drs. Furchgott, Ignarro, and Murad in recognition of their work establishing that nitric oxide (NO) is a key signaling molecule in the cardiovascular system. Ignarro linked Murad’s work on the vascular effects of exogenously applied NO to Furchgott’s classic vascular ring experiments in which he demonstrated the presence of an endothelially derived relaxing factor. Subsequently, it has become apparent that this outwardly simple diatomic free radical gas modulates a wide range of biological activities including vascular homeostasis, neurotransmission and host defense; and is implicated in the pathogenesis of many disease states as diverse as arteriosclerosis and Alzheimer’s disease.

BIOSYNTHESIS OF NITRIC OXIDE

Production

NO is formed stereo-specifically from the terminal guanidine nitrogen of the semi essential amino acid L-arginine, catalysed by a family of hemoproteins, the NO synthases (NOS). NOS has both oxidoreductase and oxygenase domains. The reductase domain binds flavin-adenine mononucleotide, flavin mononucleotide, and nicotinamide adenine dinucleotide phosphate (NADPH). The oxidase domain binds L-arginine, a heme moiety (iron
protoporphyrin IX), and tetrahydrobiopterin. Homo-dimerization of the enzyme allows trans transfer of electrons from NAPDH to the oxidase domain, converting L-arginine to NO and L-citrulline, via N-hydroxy-L-arginine. The process consumes molecular oxygen.

Three isoforms of NOS, with 50-60% sequence homology, have been identified and subsequently cloned: neuronal (nNOS); endothelial (eNOS); and inducible (iNOS) or types 1, 2, and 3 respectively [1]. nNOS is encoded on chromosome 12, and is constitutively expressed in neurones, skeletal muscle, renal mesangium and pancreatic islets. eNOS is encoded on chromosome 7, and is constitutively expressed in the membrane and Golgi apparatus of endothelium, platelets, endocardium, and myocardium. iNOS is encoded on chromosome 17 and, except in the renal mesangium, is not generally expressed under physiological conditions. Immune cells, vascular smooth muscle, endothelial cells, myocardium, hepatocytes, astrocytes, renal mesangial cells, chondrocytes and fibroblasts can all be induced to express iNOS, which lacks the specific membrane targeting sequence of the other isoforms and is thus found in the cytosol. The presence of NOS in rat mitochondria (mtNOS) has been demonstrated [2], although whether this is a distinct nuclear encoded isoform or a post translational modification of type 2 NOS is unclear [3]. mtNOS has a role in the regulation of respiration, matrix pH, and transmembrane potential in mitochondria.

**Regulation of NOS Activity**

All three NOS isoforms have comparable specific activities in the presence of appropriate calcium levels (vide infra). Enzymatic output is therefore rate limited by substrate availability and the quantity of enzyme present. All three isoforms rely on the presence of calmodulin, which facilitates the transfer of electrons through the enzyme. For nNOS and eNOS, calmodulin binding occurs in response to intracellular calcium transients of the order of ~100 nM. These transients may result from activity of agonists such as bradykinin, acetylcholine, glutamate, or from changes in vascular shear stress. Constitutive NO production is rapid and short-lived and in the picomolar range. eNOS is also regulated at a transcriptional level by vascular shear stress, exercise training, chronic hypoxia, and heart failure. Calmodulin binding in iNOS unusually requires very low levels of calcium (30-70 nM); enzymatic activity is thus regulated at a transcriptional level. The promoter region of the human iNOS gene contains the appropriate response elements for shear stress and interferon-γ (IFN-γ), and a nuclear factor kappa B (NF-κB) binding consensus sequence [4]. In murine models, NF-κB activation is