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Guest Editorial

Nitrogen Monoxide in Health and Disease

It has been known for long that bacteria produce nitrogen monoxide (NO). But one could hardly imagine even a decade ago that this molecule is also a highly significant regulator involved in higher organisms. This small molecule is involved in various physiological activities, such as immune regulation, platelet inhibition, regulation of blood pressure, penile errection and even memory (1, 2). Surely, the list is likey to grow with time.

The story began in the early 1980s in different areas of biomedical research almost simultaneously and rather silently. When stimulated with norepinephine, blood vessels constrict because vascular smooth muscle cells have specific receptors for norepinephrine. Acetylcholine, on the other hand, causes relaxation of vascular smooth muscle cells. However, it was observed that acetylchoine-dependent vascular relaxation was not seen in vessels denuded of endothelium (3). Indeed, endothelial cells reportedly have receptors which interact with acetylcholine to produce a small molecule of very short biological half life termed as endothelium-derived relaxing factor (EDRF). EDRF acts on vascular muscle cells and induces relaxation. Now it appears that EDRF is actually NO. Nitroglycerine, which is used in angina, is itself hardly effective. However, it is metabolically converted into NO, which in turn relaxes vascular smooth muscles (4). Researchers are now attempting to delineate the association between NO metabolism and blood pressure.

The most startling and relatively recent aspect of NO is its involvement in brain functions. Glutamate is known to be an excitatory neurotransmitter. Its action is mediated by an increase in calcium influx and cyclic guanosine 3' 5' -monophosphate (cGMP) production. Interestingly, one rate limiting factor in the process of glutamate-mediated neural excitation is L-arginine. The link between arginine and cyclic GMP production is now known to be NO. The scenario is relatively simple and seems to be nearly complete. Glutamate affects various subtypes of receptors : one of them is the N-methyl-D-aspartate (NMDA) receptor. Activation of this receptor opens ion channels to promote calcium influx, calcium ions form a complex with calmodulin. The calcium-calmodulin complex activates an enzyme, nitric oxide synthase (NOS). Activated NOS produces NO from the guanidino nitrogen atom of L-arginine through two sequential monooxygenase reactions (5,6). NO acts on the enzyme guanylyl cyclase, specifically on the iron moiety of the active site of this enzyme leading to conformational change in the enzyme molecule (reminiscent of oxygen binding to haemoglobin!). The activated guanylyl cyclase in turn produces cGMP from guanosine triphosphate (GTP). There is evidence to suggest that this pathway is involved in the process of long term potentiation. Similarly, other target cell reactions including vasodilation and platelet inhibition are also mediated by the action of NO on guanylyl cyclase-mediated increases in cGMP (1,2,4).

Since early 1980s, it has been known that dietary nitrates are not the sole source of nitrate in the human body, and that nitrate increases in inflammatory states and in response

to bacterial endotoxins. Macrophage deficient mice, however, fail to show this response to the fullest extent. Now we know that macrophages, when activated by endotoxin or T cells, metabolize L-arginine to produce NO (7). NO is highly reactive and attacks tumour cells, bacteria and other foreign bodies. A specific blocker, methyl derivative of arginine, however inhibits this macrophage reaction.

A whole gamut of new question is now open for research. Most important is the issue of functional dichotomy of NO. While on one hand NO acts as a signal for neural activity, vascular tone and other physiological activities, it also mediates toxic actions of macrophages. There is evidence that NOS-positive neurons may often be NADPH-diaphorase positive as well, and that these are resistant to neuro-degenerative losses commonly associated with a few diseases. Why does then excess stimulation with glutamate induce toxic neurodegeneration? This degenerative change can be blocked by using an NOS inhibitor, or by the lack of arginine in *in vitro* studies, or by neutralizing NO with haemoglobin. Clearly, the toxic effect of glutamate involves NO. According to a recent hypothesis (8), the apparent functional dichotomy resides in the regulation of NOS by calmodulin. In endothelium and in neurons the activity of constitutive NOS is modulated by calmodulin. In activated macrophages, on the contrary, NOS is inducible and the enzyme contains activated calmodulin regulator as a subunit. In other words, NO production in endothelium and neurons is regulated by a servomechanism, while its production/secretion by activated macrophages is always maximal. Does it mean that maximal stimulation of NOS relates to the observed toxic activity of NO? Can we say that over-stimultated NMDA neurons secrete NO like macrophages, and thus kill adjacent neuronal cells? What could be the paraneural function of nitrinergic signal transduction (9)? Are polyamines involved in the process of NMDA receptor-mediated excitotoxicity (10)? Questions are piling up.

It is however, possible that the explanation of differential functions of NO resides in its chemistry. Under physiological and pathological conditions NO appears in at least three possible bioactive and interconvertible forms : nitric oxide ($NO^{\bullet}$), nitrosonium cation (NO^{+}) and nitroxyl anion (NO^{-}). It has been suggested that the existence of various subtypes depends on the redox state of NO pools and that the form in which NO is present may affect its biological actions. For example, $NO^{\bullet}$ reacts with the haeme moiety of haemoglobin. Its NO^{+} form primarily reacts with amine, whie NO^{-} form reacts with sulfhydryl residues. Thus the action of NO may depend upon the form in which it is delivered and transported at the target site. This may provide a clue to solve the apparent paradox of protective and toxic actions of NO in different cell systems (11). Further research is needed to understand the physiological and pharmacological chemistry of NO so that it can be tailored for therapeutic use.

The production and action of NO are very rapid, making it a tough game mate. Though it is now possible to detect NO release from single cells, localization of the NOS-positive cells by immunocytochemistry and the presence of NOS signal by *in situ* hybridization are more practical. NOS is an interesting biomolecule. It is generally present as a dimer. It has a binding site for calmodulin and bears recognition sites for reduced nicotinamide adenine dinucleotide phosphate (NADP) and flavins (FMN and FAD). It uses tetrahydropterin and thiol residues as cofactors. NOS shows striking amino acid sequence homology with cytochrome P-450 reductase. Like P-450, NOS also contains iron-protoporphyrin IX (7,12,13).

It also has residues for phosphorylation which are modulated by protein kinases. All these facts provide handles for study of NOS, which in turn is likely to teach us more about NO, and its role in the action of hormones, neurotransmitters, growth factors and other cytokines.

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