Nitric oxide (NO) is a unique, endogenous regulatory molecule that is involved in a wide variety of physiological processes in multiple organ systems. This simple gas functions as a cellular messenger in a broad range of biological activities that include blood pressure regulation, immunomodulation and neurotransmission. It has also been implicated in a number of homeostatic functions in the cardiovascular system: it is a significant determinant of basal vascular tone and, in addition is thought to regulate myocardial contractility and platelet aggregation. Dysregulation of NO mediated effects have been implicated in the pathogenesis of essential hypertension, atherosclerosis, and the hypotension associated with septic shock. This review will focus on these multiple effects of NO in the cardiovascular system.

NO Synthesis.

NO is synthesised within different cells, including the vascular endothelium, macrophages, platelets, and neuronal cells. The terminal guanidino nitrogen atom(s) of the amino acid L-arginine has been shown to be the substrate from which NO is derived in vascular smooth muscle cells, macrophages and endothelial cells. This reaction is catalysed by the enzyme NO synthase (NOS). Three NO synthases have been described: an endothelial isoform (eNOS), a neuronal isoform (nNOS) in >nitrergic= nerves and a macrophage or inducible isoform (iNOS). The genes encoding for the three isoforms of NO synthase have been located to human chromosome 7 (eNOS), 12 (nNOS) and 17 (iNOS). The two predominant isoforms of NOS are eNOS and nNOS. eNOS is the constitutive
enzyme found in the vascular endothelium where it generates a basal level of NO that regulates vascular tone. iNOS is the inducible enzyme that is activated by exposure to bacterial endotoxin or cytokines such as IL-1β and TNFα. iNOS has been found in macrophages, neutrophils and vascular smooth muscle cells.

**NO, Immunity and Inflammation.**

It is now clear that activated macrophages express high levels of NOS and produce large amounts of NO. Cytokines that induce the expression of iNOS in macrophages include interleukin-1 (IL-1), interferon-gamma (IFN-gamma), tumour necrosis factor-alpha (TNF-alpha) and migration inhibitory factor. The NO generated contributes to the role of macrophages as highly effective killers of intra- and extracellular pathogens (although NO itself is probably not the cytotoxic molecule, rather it may be peroxynitrite (ONOO⁻) a product of the interaction between NO and oxygen).

It is also apparent that excessive NO can contribute to immunopathology (diabetes, graft-v.-host disease, liver cirrhosis, rheumatoid arthritis) and expression of iNOS is thus necessarily under tight regulation. A number of cytokines, including IL-1, IL-10 and transforming growth factor-beta, can down regulate the induction of NO synthase in macrophages. NO itself can reduce the activity of NO synthase by feedback inhibition, and also inhibit the production of IFN-gamma by Th1 cells (thus turning off its own synthesis from upstream in the inflammation cascade). It is also clear that iNOS expression is involved not only in macrophage biology, but also in the response of a wide range of other inflammatory cells.

**NO and the Cardiovascular System.**

**Physiological effects of NO.**

Peripheral vascular system: eNOS derived NO is involved in the regulation of vascular tone. Inhibition of NO synthesis in healthy volunteers causes substantial arteriolar vasoconstriction and an increase in blood pressure, suggesting that the tonic NO-mediated dilatation of resistance vessels may be counterbalancing the sympathetic nervous system. Moreover, recent studies in genetically modified mice lacking the gene encoding for eNOS (eNOS knockout mice) have shown they develop hypertension. These two systems (the NO and sympathetic nervous system) might therefore work together to allow vascular tone to be adjusted rapidly in response to a variety of central and local stimuli; the sympathetic nervous system providing a mechanism for central control and endothelium-derived NO a system for local control.

NO appears to play an important role in the distribution of blood flow in vascular networks. Resistance and conduit vessels dilate in response to increases in blood flow, and this vasodilatation appears to be secondary to shear stress induced NO release. NO may also regulate blood flow distribution among other arteriolar branches.

Heart: NO has recently been shown to have a direct regulatory effect on myocardial contractility. Shultz et al reported evidence of both iNOS and eNOS in adult rat myocytes and ventricular tissue after exposure to cytokines or endotoxin. Balligand et al have also shown that an endogenous NO signalling pathway regulates the responsiveness of ventricular myocytes both to adrenergic and cholinergic contractility. These observations suggest that eNOS activity in the heart might regulate the countervailing effects of sympathetic stimulation and its counter-regulatory role in the periphery. The role of iNOS in humans is more controversial with some studies reporting the presence of iNOS in myocytes whilst other studies reporting none. The precise role and effects of NO expression therefore remains to be determined. Indeed, it is possible that the NO is produced primarily as a host defence mechanism, and that the cardiac or vascular changes are secondary.

NO and platelet function: NO released under basal conditions (eNOS) also plays an important role in regulating platelet-vessel wall interactions. It inhibits platelet adhesion by stimulating soluble guanylate cyclase and increasing cyclic guanosine monophosphate; this leads to reduced platelet aggregation. It also interacts synergistically with prostacyclin to modulate platelet aggregation and although prostacyclin is a very powerful endogenous inhibitor of platelet aggregation, NO has the additional effect of inhibiting platelet adhesion, an effect that prostacyclin does not possess.

**Pathophysiological effects of NO.**

NO plays a major role in maintaining vascular homeostasis and therefore it is not surprising that dysregulation of NO production has widespread effects. NO synthesis has been shown to attenuate myocyte contraction and contribute to the hypotension in septic shock. By contrast, it has been suggested that dysfunctional NO metabolism may be involved in the pathogenesis of essential hypertension.

A defect in eNOS has also been implicated in the pathogenesis of atherogenesis and vasospasm. Basal NO release maintains the integrity of the endothelium by opposing local platelet-induced aggregation and vasoconstriction and inhibits foam-cell formation and migration. Endothelial damage, whether secondary to angioplasty or to atherosclerosis, leads to an impairment in NO production. This reduction in NO release in an area of injured endothelium may lead to unopposed platelet aggregation, vasoconstriction and leukocyte infiltration, all of which contribute to the process of atherosclerosis.

Excess NO production might be involved in the pathogenesis of both septic shock and myocardial stunning (following reperfusion of ischaemic myocardium). Indeed the profound vasorelaxant effects of NO are thought to partially explain the blunted vasoconstrictor response to adrenergic agents in septic shock. Vallance et al have shown that the NOS inhibitor (N⁵-monomethyl-L-arginine) used in septic shock can cause a rapid, transient increase in blood pressure and a return of vascular responsiveness to conventional vasoconstrictors like noradrenaline.

**Cardiovascular therapeutics.**

L-arginine supplementation: Supplementation with L-arginine, the substrate for the NO pathway, appears to improve coronary endothelial function in patients with angina and restore coronary endothelial function in cardiac transplant patients. In a recent study in patients with heart failure, chronic L-arginine supplementation was found to improve systemic blood flow. How arginine supplementation works is unclear because although L-arginine is the substrate for NO, the amount of arginine required by the enzyme is in the order of 1 μM and the circulating concentration is in the
order of 100 μM (i.e. the supply of arginine should not be rate-limiting for NO generation). One possibility is that the presence of an endogenous inhibitor of NOS (such as asymmetric dimethylarginine; ADMA) competes with arginine for the active site. Studies are now underway to explore this and other possibilities.

NO replacement therapy: The metabolism of nitrovasodilators such as nitro-glycerine and sodium nitroprusside has been extensively investigated. Both these agents are pro-drugs for NO and activate guanylyl cyclase. Nitro-glycerine is metabolised to form NO, and nitroprusside releases NO directly. Nitrovasodilators not only relax vascular smooth muscle, but, like NO itself, inhibit platelet aggregation. Folts et al have also demonstrated that intravenous nitro-glycerine inhibits platelet aggregation in a model of coronary stenosis. Although ISIS-4 showed no effects of nitrates on mortality post myocardial infarction, a recent meta-analysis, showed that nitrates used in the setting of an acute myocardial infarction lead to a 35% reduction in mortality. This remarkable finding suggests that the antiplatelet effects of nitrates may be more significant therapeutically than previously believed. There is also evidence that NO or exogenous NO donors may be useful in preventing the vascular smooth muscle cell proliferation that occurs after angioplasty. Restenosis is a major problem that occurs in 30 to 40% of patients after angioplasty. In animal models of angioplasty-induced endothelial injury, the damaged endothelium loses its ability to generate substances like NO resulting in unopposed platelet aggregation and platelet-induced vasoconstriction. Platelet activation with release of growth factors is followed by smooth muscle proliferation five to seven days thereafter. There is evidence that nitric oxide-generating vasodilators can inhibit this vascular smooth muscle mitogenesis and proliferation.

Recent investigative work has focused on the development of new classes of nitrovasodilators. GSNO has recently been shown to have selective anti-platelet effects. It is able to inhibit platelet activation following angioplasty at doses that have minimal effects on arteriolar tone. Nitrosothiols, including GSNO have been shown to occur endogenously and this raises the possibility that not only the stability of endogenous NO, but also its profile of activity on different tissues could be altered by interaction with thiols. The challenge is now to develop more selective NO donors that have specific effects on platelet, vascular or cardiac function thereby avoiding the current adverse effects of excessive vasodilatation experience with the currently available nitrates. Another exciting prospect is the recent work on direct activators/inhibitors of guanylate cyclase, the enzyme responsible for production of cyclic guanosine monophosphate, which is ultimately responsible for causing smooth muscle relaxation and inhibition of platelet aggregation.

NO inhibitors: Inhibitors of NO might also have therapeutic utility in conditions such as septic shock. Indeed the non-selective NOS inhibitor L-NMMA is already in clinical trials for the treatment of septic shock. However selective inhibitors of the three isoforms of NOS might further clarify the role of NOS in disease states and should provide greater and more therapeutically effective use of these agents.

References


