REVIEW

Nitric oxide

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Nitric oxide is a gas generally known by its chemical formula NO, or NO•. The dot denotes an unpaired electron, which is the definition of a free radical; possessing this, NO is highly reactive and quite different from the anaesthetic agent nitrous oxide (N_2O), which is extremely stable. Until recently, NO was best known as a constituent of car exhaust fumes, contributing to the photochemical smog of cities such as Los Angeles. In contrast, the work which crucially altered this perception was performed in a leafy Kentish suburb at the Wellcome Foundation in Beckenham, UK. In 1987, it was shown that NO was the long sought endothelium derived relaxing factor (EDRF).[1] This was a crucial discovery for cardiovascular biology, and it soon became evident that NO was produced by many cell types and performed diverse functions, including inhibition of platelet aggregation and mediation of the cytotoxic action of activated macrophages, and had a role in central and peripheral neurotransmission.

NO is enzymically synthesised from L-arginine by oxidation of one of the terminal guanidino nitrogen atoms of L-arginine,[2]— a process inhibited by L-arginine analogues such as N^G-monomethyl-L-arginine (LNMMA). Figure 1 illustrates the mechanism by which NO mediates vasodilatation. NO is synthesised within the endothelial cell and released in response to vasodilator stimuli such as acetylcholine and bradykinin. Released NO diffuses to adjacent vascular smooth muscle cells (VSMC), where it activates guanylate cyclase (GCase) so increasing cyclic GMP (cGMP) concentrations. Increased intracellular cGMP concentrations cause relaxation of VSMC, resulting in vasodilatation. However, the key to understanding the diverse roles of NO is that NO synthases (NOS) generate NO by both constitutive and cytokine/endotoxin induced pathways.

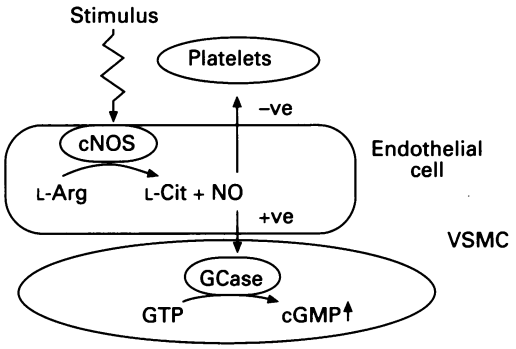

Figure 1 Mechanism of nitric oxide (NO) mediated vasodilatation in response to various stimuli such as bradykinin or acetylcholine. NO diffuses into adjacent vascular smooth muscle cells (VSMC), activating guanylate cyclase (GCase), increasing cGMP levels, and producing VSMC relaxation and vasodilatation. In contrast, NO inhibits platelet adhesion and aggregation. cNOS = Constitutive NO synthase; L-Arg = L-arginine; L-Cit = L-citrulline.

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Accepted for publication 31 August 1995

Enzymology—constitutive and inducible NO synthases

The original description of endothelial cell NO generation involved the *constitutive* enzyme (cNOS),[1 2] which is also present in the adrenal gland,[3] platelets,[4] fibroblasts,[5] polymorphonuclear leucocytes (PMN),[6] brain,[7 8] retina,[9] and some non-adrenergic non-cholinergic nerve terminals.[10] The *inducible* enzyme (iNOS) is present in many cells including endothelial cells,[11 12] VSMC,[13] macrophages,[14 15] PMN,[16] lymphocytes,[17] fibroblasts,[5] hepatocytes,[18] mast cells,[19] renal mesangial cells,[20] rabbit articular chondrocytes,[21 22] and rabbit synovial fibroblasts.[23] NO synthesis by cells in which iNOS has been induced is *several orders of magnitude greater* than in cells producing NO via cNOS.

The NO synthases require NADPH, tetrahydrobiopterin (BH_4), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and haem as cofactors, but the constitutive enzyme is Ca^{2+}/calmodulin dependent and the inducible enzyme is Ca^{2+}/calmodulin independent. The cerebellar cNOS was the first to be isolated[24 25] and cloned,[26] revealing a 1433 amino acid 160 kDa protein. The cell membrane located endothelial cNOS (both bovine[27–29] and human [30 31]) are about 130 kDa and exhibit only 58% sequence homology with rat cerebellar cNOS,[27] compared with the 51% homology between rat cerebellar cNOS and mouse macrophage iNOS[32] which, like other cellular iNOS, is a cytoplasmic protein of about 130 kDa.[32–36] The notations 'eNOS' and 'nNOS' have been used for the cNOS of endothelial and neuronal cells, respectively, though neither is confined to its respective cell type. The genes for the human eNOS, nNOS, and iNOS are located on chromosomes 7, 12, and 17 respectively.[37–40] Cloning[26] and other studies[41] also suggest that NOS are cytochrome *P*-450-type haemoproteins. Essentially, these function by transferring electrons via flavin moieties to haem, and it is likely that FAD/FMN fulfil a similar role in relation to NOS during the oxidation of L-arginine to NO. It is also clear that the tight binding of calmodulin to iNOS explains its Ca^{2+}/calmodulin independence,[42] that cNOS phosphorylation via several protein kinases may be an important influence on NOS

activity,[43] and that the similarity between the NOS haem moiety and that of GCase represents a mechanism by which NO might achieve feedback inhibition of NOS.[44]

Cytokine induction

The best recognised inducers of iNOS are interferon gamma (IFNγ), tumour necrosis factor α (TNFα), interleukin-1 (IL-1), and lipopolysaccharide (LPS)/endotoxin.[12 13 20-23] Induction of iNOS is suppressed by TGFβ, IL-4 and IL-10 alone,[45 46] and synergistically in macrophages,[47] whilst IL-8 inhibits iNOS induction in PMN.[48] The inducer and suppressor cytokines noted above correspond to those secreted by the Th1 and Th2 subsets of CD4 positive T cells (see below: Immune system). Glucocorticoids also inhibit the induction, but not the activity, of the inducible enzyme.[11] The cytotoxic potential of NO demands strict regulation of iNOS expression, thus generation of significant quantities of NO requires a specific order of stimulation by at least two agents.[49] This is illustrated by the synergy between IFNγ and LPS which, respectively, were recognised as primer and trigger of various macrophage functions, including cytotoxicity, before NO was shown to mediate the latter. Mouse macrophages produce similar quantities of NO when costimulated with IFNγ and LPS or IFNγ followed by LPS, but LPS followed by IFNγ yields much less NO[49] in proportion to the length of preincubation with LPS.[50] NO production is also sustained by continued stimulation with LPS, but not IFNγ.[49] Regulation of iNOS expression may occur at many levels, though the synergistic effect of IFNγ and LPS is principally transcriptional.[51] Recent studies of the promoter region of the mouse iNOS gene have identified putative binding sites for several transcription factors,[52] including that of an IFNγ responsive transcription factor critical to the synergy between IFNγ and LPS.[53]

Cellular effects and mechanisms

The function of nitric oxide as an intercellular messenger is enhanced by its low molecular weight, high diffusibility, and lipid solubility, whilst the cellular effects of NO owe much to its reactivity as a free radical. The best described interactions are those with iron containing proteins and the superoxide anion (O_2^-). The principal action of constitutive NO is activation of GCase by binding to the haem group of the enzyme; this is believed to result from breakage of the haem-protein bond induced by NO-haem binding. The result is an increase in intracellular cGMP, achieving smooth muscle cell relaxation principally by decreasing intracellular Ca^{2+}. Conversely, cytokine induced NO production mediates cytotoxicity in the target cells of macrophages by inhibiting non-haem iron-sulphur centred enzymes and releasing iron.[54] Key iron-sulphur (Fe-S) enzymes inhibited by NO synthesis include aconitase, the rate limiting enzyme of the citric acid cycle, complexes I and II in the mitochondrial electron transport chain, and ribonucleotide reductase which synthesizes the deoxynucleosides required for cell replication.[14 54] In contrast, NO also inhibits the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase by stimulating its ADP-ribosylation.[55]

The above information suggests that substantial NO induced increases in intracellular free iron concentrations occur directly as a result of NO releasing iron from both iron containing enzymes[56] and ferritin.[57] However, this may be mediated by NO via an Fe-S containing protein (iron regulatory factor (IRF)) involved in cellular iron homeostasis. IRF binds to iron responsive elements in the mRNAs of ferritin and the transferrin receptor. In iron replete cells, iron responsive element binding by IRF is low, whereas in iron poor cells such binding by IRF increases, impairing iron storage via ferritin. NO increases iron responsive element binding by IRF,[58 59] decreasing the biosynthesis of ferritin, which should lead to the observed increases in intracellular free iron levels. The latter would also enhance the generation of reactive oxygen species via the Fenton and Haber-Weiss reactions.

The interaction of NO with O_2^- is quite different from that with iron, and is one of several possible pathways for NO breakdown in vivo. The result is the elimination of both radicals.[60] In the vasculature, this would abrogate the vasodilator and antithrombotic properties of NO, but also remove O_2^-, which is implicated in ischaemic reperfusion injury of both the myocardium[61] and the synovium.[62] Under some circumstances the above mechanism would appear homeostatic were it not that the reaction product, the peroxynitrite anion ($ONOO^-$), is a powerful oxidising agent that may also yield the hydroxyl radical.[63 64] Whilst contributing to the cytotoxicity of activated macrophages and neutrophils, formation of peroxynitrite and the hydroxyl radical may also mediate tissue injury in hypoxic-reperfusion injury, acute inflammation and other situations generating NO or O_2^-.

In aqueous solution, NO is spontaneously oxidised by oxygen to nitrite (NO_2^-), which in ex vivo studies is the major metabolite of NO in the coronary circulation.[65] However, in vivo NO_2^- may be further oxidised by erythrocyte haemoglobin to nitrate (NO_3^-) which is also the end product of the interaction of NO and O_2^-. The half life of NO is between three and 50 seconds, but may be much longer depending on ambient NO, oxygen and O_2^- concentrations.[1] The half life may also be extended physiologically by NO forming nitrosoproteins with vasorelaxant properties attributable to NO release,[66] enabling NO to exert effects at distant sites.

Below, we briefly consider actions of NO in the cardiovascular, nervous and immune systems that help to illuminate the pathophysiological relevance of NO to patients with rheumatic diseases including multisystem disorders.

The cardiovascular system

The simplified representation of endothelium dependent vasodilatation by NO (fig 1) shows that NO is released by endothelial cells in response to several agonists. NO is the major influence on basal vascular tone in vitro and in vivo,[67] including that of the coronary circulation.[68] NOS inhibitors such as LNMMA produce vasoconstriction in both the arterial and venous systems including the microcirculation, with the most marked effects evident in large arterial resistance vessels,[69] though these effects do vary between species. In man, NO mediates both basal and stimulated arterial vasodilatation and stimulated venodilatation.[70 71] Conversion to NO or direct release of NO is also the mechanism by which organic nitrates exert their beneficial cardiovascular effects.

Thus NO is anticipated to have a central role in the endothelial dysfunction and pathophysiology of atherosclerosis and essential hypertension. The former is a complex process that may have multiple effects on coronary endothelial NO synthesis-release and vice versa. Vasorelaxation is impaired in early atherosclerosis,[72] and recent studies of coronary artery explants show that basal and stimulated NO release are impaired in established disease, not just at sites of atheroma but throughout the vessel, implying generalised endothelial dysfunction.[73] Cholesterol, and especially its low density lipoprotein fraction (LDL), may be responsible for such dysfunction, as both are known to impair endothelium dependent vasodilatation and NO release,[74] whilst L-arginine (the precursor of NO) reverses lipid induced enothelial dysfunction in both animals[75] and man.[76] Lipids may exert this effect via impairment of endothelial cell NO synthesis, though inactivation of NO by oxidised LDL[77] and LDL interactions with guanylate cyclase[78] may also be important. However, impaired vasodilatation is only one consequence of the presence of decreased amounts of NO in the endothelial milieu. NO has the potential to inactivate O_2^- which would otherwise oxidise LDL[79]—a modification proposed as crucial in atherosclerosis as it promotes the uptake of LDL by macrophages.[80] Generation of reactive oxygen species is also presumed to be a principal factor in NO mediated hypoxic-reperfusion injury.[81 82]

NO inhibits platelet adhesion,[83] the key step in thrombosis in addition to platelet aggregation which results in the release of potent vasoconstrictors and mitogens. In vivo NO is crucial in opposing platelet induced vasoconstriction[84] caused by agents such as thromboxane; however, other platelet products such as serotonin and ADP/ATP also stimulate endothelial cell NO release, opposing vasoconstriction, and inhibit platelet function in tandem with platelet derived NO[4] and prostacyclin.[85] Platelet derived mitogens are also the presumed cause of VSMC proliferation characteristic of atheroma, which NO antagonises directly via an antiproliferative effect on VSMC.[86] The significance of negative platelet regulation by organic nitrates in clinical usage is debatable. Mediated via NO, nitrates are, of course, potent vasodilators, though their long term efficacy is limited by the development of tolerance, the mechanism of which remains controversial. Explanations include depletion of intracellular thiols essential for the conversion of organic nitrates to NO in addition to the regulation of GCase[87] and desensitisation of GCase to NO[88] making VSMC less responsive to nitrates.

Impaired NO dependent vasorelaxation is evident in both animal models of hypertension and hypertensive patients.[89] Moreover, orally administered NOS inhibitors produced sustained hypertension for the duration of studies lasting five to eight weeks.[90 91] Potential mechanisms include a central nervous system (CNS) action[92] or alterations in renal homeostasis, as there is increasing evidence that NO has important actions in the kidney. NOS inhibitors cause renal vasoconstriction, a decrease in GFR, and an increase in glomerular capillary pressure,[93] whilst increased urinary nitrite (NO_2^-) and nitrate (NO_3^-) in response to a high salt intake implicate NO in the renal mechanisms concerned with regulating blood pressure.[94] This is supported by data that NO influences perfusion pressure control of renin secretion[94] and that low doses of NOS inhibitors cause volume dependent hypertension.[95] Impaired NO synthesis is also implicated in the hypertensive response of salt sensitive Dahl/Rapp rats fed a high salt diet—an effect both prevented and reversed by feeding L-arginine.[96]

The nervous system

Research to date has focused on the constitutive nNOS present in both the central and peripheral nervous systems, although iNOS has been described.[97] NO synthesis, originally described in the forebrain and cerebellum, is evident throughout the brain,[98] including the cerebral cortex, corpus striatum, midbrain, hippocampus, and pituitary. Moreover, immunological and in situ RNA hybridisation studies have shown that nNOS is responsible for the activity of neuronal NADPH diaphorase—a well established histochemical stain.[99 100] This is important, because NADPH diaphorase activity (and thus nNOS) is present in only a small percentage of neurones that are particularly resistant to degeneration in Huntington's chorea,[101] hypoxia,[102] and neurotoxicity caused by excitatory amino acid neurotransmitters.[103] Principal among the last of these is glutamate, which, acting via stimulation of the postsynaptic *N*-methyl-D-aspartate receptor (NMDA), is a proven mediator of neurotoxicity in models of stroke and a candidate mediator in neurodegenerative disorders. NMDA receptor activation may achieve different cellular effects via receptor diversity, but the most prominent effect is a Ca^{2+} influx, which would be expected to increase neuronal Ca^{2+} concentrations sufficiently to activate the Ca^{2+} dependent nNOS. Experimental evidence from several groups has confirmed that NO mediates glutamate neurotoxicity in vitro[104]

and in vivo.[79 105 106] The neuroprotective effect of superoxide dismutase implicates peroxynitrite or its decomposition products.[107] However, some NO releasing compounds are neuroprotective,[107] supporting the proposal that the redox state of NO determines toxicity.[108] It is suggested that while $NO^{\bullet}$ is neurotoxic, NO^{+}, the nitrosonium ion, is neuroprotective. A plausible explanation is that NO^{+}, by reacting with thiol groups, nitrosylates the NMDA receptor, thus blocking NMDA receptor neurotoxicity mediated by $NO^{\bullet}$. Clinical trials are currently evaluating NMDA receptor antagonists in acute stroke, but inhibition of NOS in a mouse model of acute stroke proved more effective in limiting neuronal damage than did NMDA receptor antagonism.[109] The evidence above suggests nNOS inhibition may have a therapeutic role in stroke and neurodegenerative disorders.

CNS neurotoxicity is clearly an aberrant pathological consequence of NOS activity that also has important physiological functions in the brain. This is particularly true in the 'molecular' layer of the cerebellum, where granule and basket cells produce NO in response to NMDA receptor activation by mossy fibres and parallel fibres, respectively.[110] Probable targets of NO are adjacent cells such as astrocytes and other glia, in addition to neurones and the GCase of the presynaptic terminal[111] (fig 2). This last possibility suggests the potential of NO to act as a *retrograde synaptic messenger*,[112] presumably modulating synaptic transmission, and stimulated the hypothesis that NO has a role in the plasticity of the CNS.[113] This is sustained by evidence that, in the cerebellum, NO mediates long term synaptic depression,[114] believed to be the cellular mechanism of cerebellar learning and analogous to the long term potentiation in the hippocampus that is implicated in learning and memory.[112] NO has been shown to enhance and NOS inhibitors to impair[115] long term potentiation in the hippocampus, whilst in vivo NO inhibition blocks spatial learning.[116] It has been proposed that NO modulates synaptic efficacy by acting as a local temporo-spatial signal, strengthening synapses that fire in a co-ordinated manner and weakening those that do not, thus linking local neuronal activity.[113] The same authors also proposed a role for NO in the development of the nervous system and the coupling of neural activity to local increases in cerebral blood flow, demonstrated by positron emission tomography (PET). Indeed, nNOS does mediate vasodilatation,[117] and NOS inhibition uncouples the increased cortical blood flow that occurs with peripheral nerve stimulation.[118 119]

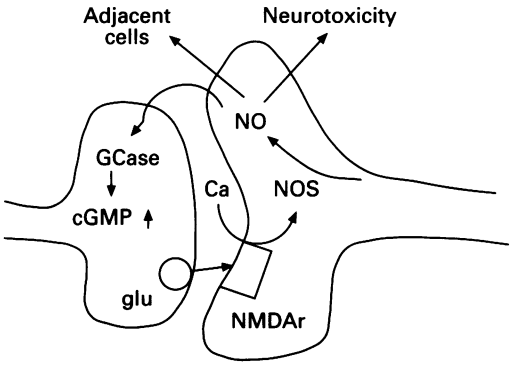

Figure 2 Model for the role of nitric oxide (NO) as a CNS neurotransmitter. Glutamate (glu) is an excitatory neurotransmitter acting on the N-methyl-D-aspartate receptor (NMDAr). NMDAr stimulation produces a postsynaptic calcium influx (Ca) resulting in NO synthesis by the calcium dependent constitutive NO synthase (NOS). NO may act as a retrograde neurotransmitter acting on the guanylate cyclase (GCase) of the presynaptic terminal to enhance neurotransmission. Diffusing to adjacent cells, it may also link and enhance coordinated local neuronal activity. There is also strong evidence that NO mediates NMDA neurotoxicity.

The elucidation of 'vascular evoked responses' using PET has advanced our understanding of pain and, specifically, its cortical appreciation. NO is a candidate mediator of such phenomenona and is implicated in pain. NO exhibits a peripheral analgesic effect via stimulation of GCase and increased cGMP concentrations[120–122] and, when administered systemically, L-arginine also has a central antinociceptive action via opioidergic pathways.[123 124] In contrast NO also mediates hyperalgesia at the spinal or supraspinal level[125–127] and is implicated in both the development of tolerance to morphine,[128] and thalamocortical function.[129] The above suggests not only an important role for NO in pain mechanisms, but that its actions may be pro- or antinociceptive at different sites.

Key sites of NO synthesis within the autonomic nervous system are non-adrenergic, non-cholinergic (NANC) nerve terminals that generally serve to relax smooth muscle. NO appears to be the principal NANC neurotransmitter, and NO mediated NANC function has been identified in the gastrointestinal tract,[130 131] corpus cavernosum,[132] the respiratory system,[133] and arterial vessels.[134] Evidence suggests that NO is the physiological mediator of penile erection,[135] modulates the effects of bronchoconstrictors,[136] and has a key role in gut sphincter control:[137] mice with an nNOS gene knockout develop normally without histological abnormalities of the CNS, but with dilation of the stomach and pyloric muscle hypertrophy similar to that of pyloric stenosis in human infants (see below).[138]

The immune system

IMMUNOREGULATION, INFLAMMATION AND RHEUMATIC DISEASES

The discovery that NO was EDRF, was soon followed by the realisation that NO was also responsible for the cytotoxicity of activated macrophages[12–14] and recognition of constitutive and cytokine inducible NOS. However, NO has a broader and more complex role in immune functions and inflammation, in which both cNOS and iNOS are relevant. Important in this respect has been the recent focus on the role of the endothelium in inflammation. At the endothelial level, the principal actions of constitutive NO synthesis are vasodilatation and inhibition of platelet aggregation. These functions are particularly relevant to both the abnormal vascular tone

associated with many inflammatory rheumatic diseases, evinced by Raynaud's phenomenon and the presence in chronic inflammatory diseases of microvascular abnormalities including microthrombi.[139] Indeed, a primary vascular pathogenesis has been proposed for both Crohn's disease[140] and rheumatoid arthritis,[139] whilst in the latter there is also evidence that exercising joints causes synovial hypoxia-reperfusion leading to further microvascular injury.[141 142]

In the circumstance of hypoxia-reperfusion, NO has the potential to mediate or modulate free radical induced injury, though another important consequence of hypoxia-reperfusion is an increase in leucocyte adherence to endothelium.[142a] In a cat model of myocardial ischaemia-reperfusion, impaired basal NO release during reperfusion accounted for the increased leucocyte adhesion observed.[143] Perfusion of normal cat mesenteric venules with NOS inhibitors produced a 15-fold increase in leucocyte adherence, but lesser increases in leucocyte emigration.[144] Further studies showed a marked increase in leucocyte adhesion, emigration, and vascular permeability 30 minutes after perfusion of N^G-nitro-L-arginine methyl ester (L-NAME), which was reduced by monoclonal antibodies to CD18, intercellular adhesion molecule-1, or P-selectin.[145] Subsequent evidence suggests that the L-NAME induced increases in leucocyte adhesion are the result of endothelial O_2^- and mast cell mediators unopposed by endothelial NO,[146] which is consistent with in vitro evidence that NO blocks histamine and platelet activating factor release by mast cells.[147 148] This is further persuasive evidence that the cNOS has an important role in stabilising the endothelium and opposing early inflammatory changes. Conversely, in rat models of immune complex mediated alveolitis and dermal vasculitis, NOS inhibitors greatly reduced indices of tissue injury.[149 150] Confirmation of NO as a principal effector mechanism of immune mediated vascular injury has therapeutic implications. Moreover, the protective effect of inhibiting iNOS in this circumstance is entirely compatible with the expected beneficial effects of constitutive NO release outlined above.

NO is also an effector and regulator of white cell function, as lymphocytes,[17] PMN[16] and macrophages[14] all possess NOS activity. As with the endothelium, the effect of NO is likely to be determined by the concentration of NO in the local milieu and thus reflect local iNOS activity, illustrated by the effect of NO on PMN and monocyte chemotaxis.[151 152] The key cytotoxic effector in PMN is O_2^- and though NO has an analogous role in macrophages, it was once thought of little relevance to PMN. In fact, phorbol myristate acetate (PMA) stimulation of the neutrophil respiratory burst generates both NO and O_2^-, though increases in NO synthesis occur at lower PMA concentrations, plateau earlier, and are generally less than those of O_2^-.[153] Purification of the iNOS in human PMN reveals an associated 22 kDa protein that generates O_2^-, whilst stimulation of the whole fraction by PMA increases O_2^- and decreases NO generation.[154] NO has also been shown to inhibit PMN O_2^- production via a direct effect on the membrane NADPH oxidase,[155] and to inactivate the O_2^- generating enzyme xanthine dehydrogenase/oxidase in macrophages,[156] suggesting negative feedback regulation of O_2^- generation by NO and vice versa. The above suggests a close regulatory relationship between NO and O_2^- and the reactive oxygen species generating potential of this interaction.

Low levels of NO have been implicated in lymphocyte activation and proliferation.[159] NO donors such as sodium nitroprusside, and to a lesser degree gaseous NO, increase lymphocyte uptake of glucose (an early event during lymphocyte activation), stimulate TNFα production and nuclear transcription factor κB (NF-κB) binding activity and enhance activity of the tyrosine kinase, $p56^{lck}$, which is implicated in lymphocyte signalling events.[157] All the above are effected via a cGMP independent mechanism, though guanylate cyclase activation by NO may be necessary for optimal lymphocyte proliferation.[158] L-Arginine depletion and NOS inhibitors also impair phytohaemagglutinin (PHA) stimulated proliferation,[158] whilst dietary L-arginine supplementation in man increases lymphocyte mitogenic responses to concanavilin A (con A) and PHA.[159] L-Arginine has also been shown both in vitro and in vivo to enhance natural killer and lymphokine activated killer activity.[160]

Paradoxically, high concentrations of NO which occur following macrophage activation suppress antigen presenting cell activity[161] and T cell proliferation. NOS inhibition produced a striking increase in PHA induced proliferation in rat spleen cell cultures, and greatly enhanced both the allogeneic mixed lymphocyte reaction and the development of lymphocyte cytotoxicity in both rats and mice.[162] Mills confirmed the observation that NOS inhibitors enhanced mitogen induced proliferative responses, that this was true for a variety of mitogens that activate different lymphocyte subsets, that macrophages were the 'suppressor' cells producing NO, and that differences between rat and mouse responses were probably quantitative.[163] Concurrent studies reinforced these findings and showed that T cell IFNγ was required to induce the NO mediated suppressive effects of mouse peritoneal macrophages.[164]

These findings are of very direct relevance to organ transplantation. In vivo studies of a sponge matrix allograft model revealed much greater concentrations of NO breakdown products (NO_2^-/NO_3^-) in allograft fluid compared with syngeneic graft fluid, whilst in vitro allogeneic graft infiltrating cells produced more NO than corresponding syngeneic cells, and only developed cytotoxicity when treated with the NOS inhibitor LNMMA.[165] In rat studies, LNMMA also promoted allospecific cytotoxicity[166] and restored depressed mitogenic responses to con A.[167] Thus macrophage NO synthesis, induced by cytokines derived from activated T cells, may effect negative feedback by suppressing T cell proliferation.

Direct evidence of NO production during allograft rejection in vivo has been provided by electron paramagnetic resonance (EPR). In rat heart allografts, rejection corresponds with the appearance of an NO signal on EPR in both blood and graft tissue, which is prevented by the immunosuppressive, FK506.[168] After small bowel transplantation, both rejection and graft versus host disease (GVHD) were preceded by increased serum NO_2^-/NO_3^- concentrations, but were prevented clinically and histologically (as was the increase in serum NO_2^-/NO_3^- levels) by treating transplant recipients with FK506.[169] Widening the range of allogeneic grafts undertaken yielded similar findings,[170] confirming NO_2^-/NO_3^- concentrations as an early marker of acute rejection or GVHD that merit further investigation in clinical practice. However, this proposal raises the questions whether NO synthesis in this context is a specific indicator of immune activation, and whether its net effect is to inhibit or promote rejection or GVHD. Whilst NO is cytostatic, limiting lymphocyte proliferation, increased NO synthesis may reflect macrophage and cytotoxic lymphocyte activity that is responsible for rejection and GVHD—a notion supported by the protective effect of LNMMA in GVHD.[171]

More evidence is also emerging in respect of the differential effects of cytokines on iNOS activity, and vice versa. As noted above, IFNγ and TNF are key inducers, and IL-4 and IL-10 key suppressors, of iNOS activity, produced by the Th1 and Th2 subsets of CD4 T cells, respectively. Activated Th1 cells produce large quantities of NO in comparison to undetectable levels in Th2 cells; however, NO synthesis by Th1 cells exerts negative feedback by suppressing their production of IFNγ and IL-2.[172] NO does not appear to have any significant effect in respect of Th2 cytokine production. Given the important proliferative effects of IL-2 on Th1 and CD8 cells, it is argued that NO prevents the over expansion of the T cell subsets that mediate immunopathology.[172] Also important in this context is that NO exerts negative feedback by inhibiting both iNOS and cNOS via the haem moiety of NOS.[173]

Inhibiting NO also modulates carrageenin and dextran induced acute inflammation in the rat[174] and ADP/bradykinin induced changes in microvascular permeability in the hamster cheek pouch model.[175] In addition, NO mediates neurogenic plasma exudation in the lungs of guinea pigs,[176] oedema formation caused by substance P[177] and, together with sensory nerves, skin inflammation caused by bradykinin.[178] NO inhibition also reduced neurogenic inflammation in adult rats, which was prevented by treatment with capsaicin in the neonatal period.[179] These findings suggest a central role for NO in neurogenic inflammation.

Insulin dependent diabetes illustrates the multifunctional role of NO in an autoimmune disease that involves T cell dependent and cytokine mediated destruction of the pancreatic islet β cell.[180] NO production by human islets is greatly enhanced by combinations of IL-1β, TNF, or IFNγ, while several studies suggest inhibition of insulin secretion by IL-1β and other cytokines is mediated by NO.[181 182] Both islet cells and macrophages have been shown to kill islet cells via NO in vitro.[183] Interestingly, cyclosporin A protects islet cells from NO cytotoxicity in vitro, but NOS inhibitors are far more effective,[184] and partially suppress the development of streptozotocin induced diabetes,[185] suggesting a therapeutic potential for NOS inhibitors such as aminoguanidine. The latter reduces diabetic vascular dysfunction in vivo,[186] though its effectiveness could in part result from direct inhibition of the formation of advanced glycosylation products that are known to inactivate NO in vitro and in vivo.

Despite the wealth of evidence above, there are few published studies measuring NO synthesis in human diseases, inflammatory or otherwise. Our studies implied increased NO synthesis in both rheumatoid and osteoarthritis,[187] whilst others have demonstrated similar findings in response to sepsis[188 189] or cytokine chemotherapy[190 191] and in biopsy material from patients with ulcerative colitis.[192 193] Using NO_2^- as an index of NO synthesis, we showed increased NO_2^- concentrations in the serum of patients with rheumatoid arthritis (RA) compared with controls, and in synovial fluid compared with serum from RA patients, implying NO synthesis within the joint.[187] This was to be expected, as NO generating cells such as endothelial cells, PMN, macrophages, and synoviocytes are found within inflamed synovium. There is, however, accumulating evidence that NOS inhibitors modulate inflammation in animal models, endorsing a role for NO in inflammatory rheumatic disorders including vasculitis. NO_2^- production by synovial tissue and peripheral blood mononuclear cells was increased in streptococcal cell wall induced arthritis and suppressed by LNMMA.[194] Importantly, this was accompanied not only by a marked reduction in the articular index during the acute inflammatory phase (days 1–6), but also by a dramatic reduction during the chronic T cell-macrophage phase of inflammation that was also evident but less marked if LNMMA was started on day 12 after induction of the arthritis. Synovial histology confirmed a reduction in the cellular infiltrate and erosions in the LNMMA treated animals. In rat adjuvant arthritis, L-NAME decreased, whilst L-arginine (the precursor of NO) increased paw swelling throughout the course of arthritis.[174] T cell proliferative responses and macrophage production of NO_2^- and acid phosphatase were decreased and increased in cells harvested from L-NAME and L-arginine treated animals, respectively. The inbred MRL-lpr/lpr mouse is a recognised model for human systemic lupus erythematosus that spontaneously develops an autoimmune disorder characterised by DNA antibodies, arthritis, nephritis, and vasculitis. Urinary NO_2^-/NO_3^- concentrations were demonstrated to be greater in MRL-lpr/lpr mice compared with other strains, and increased with the onset

of nephritis.[195] Administration of LNMMA reduced not only urinary NO_2^-/NO_3^- levels, but also histological indices of both arthritis and renal disease, including vasculitis and proteinuria. These findings are consistent with studies of rat models of immune complex mediated alveolitis and dermal vasculitis in which NOS inhibitors greatly reduced indices of tissue injury.[149 150] The above observations provide strong evidence for a major role of NO as a mediator of inflammatory joint disease and connective tissue disorders.

Because of its role as the regulator of basal vascular tone and principal vasodilator, one would anticipate an important role for NO in the regulation of blood flow to the joint. Indeed, inhibition of NOS by intra-arterial infusion of L-NAME substantially reduced basal blood flow and increased sympathetic vasoconstriction in both normal and inflamed rabbit knees.[196] Moreover, the reaction of NO with O_2^- to form peroxynitrite makes NO very pertinent to synovial hypoxic reperfusion injury. Such injury occurs because, in the presence of even a modest effusion, joint exercise leads to intra-articular pressure increases that exceed synovial capillary perfusion pressure, impairing synovial blood flow.[141] We investigated this in vivo by measuring synovial fluid NO_2^- levels before and after exercise (10 minutes walking) or rest (controls) in 18 patients with active RA, and found a significant decrease of 30% in mean synovial fluid NO_2^- concentrations after exercise, compared with a non-significant increase of 13% noted for control patients who were rested (unpublished data). The decreases in synovial fluid NO_2^- were more striking and persistent in patients with clinically and biochemically very active disease. These findings suggest synovial NOS activity may be impaired during joint exercise. Probable explanations include hypoxia or transmural pressure increases during exercise that inhibit endothelial NO production in some vessels,[142] and decreased shear stress as a result of impaired flow; shear stress generally stimulates endothelial NO production. However, these factors may only partially account for exercise induced decreases in synovial fluid NO_2^-, as new evidence suggests an alternative explanation with quite different implications. Zweier *et al* used an oxygen stable ferrous iron spin trap to estimate NO concentrations in an ex vivo model of cardiac ischaemia-reperfusion.[197] They found that, during ischaemia, NO may be generated non-enzymatically by the reduction of NO_2^- to NO, and that the formation of NO correlates with increasing duration of ischaemia and decreasing tissue pH, and is responsible for impaired myocardial contractility. It is probable that a similar phenomenon occurs in the synovium during joint exercise and may be responsible for the observed exercised induced decreases in synovial fluid NO_2^-. Clearly, this last possibility requires confirmation, preferably by direct measurement of NO in vivo. The implications for the pathogenesis and persistence of rheumatoid synovitis are profound, as increased NO formation could have many adverse consequences, either directly or as a result of greater NO concentrations increasing radical generation via the reaction with O_2^-.

Studies in primary knee osteoarthritis (OA) showed increased NO_2^- concentrations in OA synovial fluid compared with OA serum, and in OA serum compared with that of age and gender matched controls.[187] Explaining the latter observation challenges current concepts of OA, whilst the former suggests generation of NO within the osteoarthritic joint, but to a lesser degree than in RA. Although synovium may be responsible, chondrocytes are an alternative source of NO.[21 22] The most effective inducers of NOS in rabbit articular chondrocytes were IL-1 and LPS, which also demonstrated pronounced synergy compared with the small increases in NOS activity attributable to IL-1 in combination with TNFα or IFNγ.[21] Recent evidence that NO mediates suppression of proteoglycan synthesis by IL-1 suggests that NO is relevant to cartilage degradation in vivo.[198] The same study showed that cytokine-stimulated increases in NO production and decreased proteoglycan synthesis by rabbit articular slices in vitro were reversed by the NOS inhibitor, LNMMA. NOS activity has also recently been demonstrated in human chondrocytes in response to IL-1β, TNFα, or LPS alone,[199] which contrasts with the multiple cytokines required to induce NOS in other cells, including rabbit chondrocytes.

The potential for NO to modulate chondrocyte metabolism and perhaps cartilage degradation in RA and OA is complemented by the effect of NO on bone. Both mouse and human osteoblasts produce NO,[200–202] with the human studies showing that osteoblast NOS expression is of the inducible, not the constitutive, type and that NO production greatly impairs osteoblast proliferation.[202] Some reports have suggested that NO inhibits osteoclast function and bone resorption,[201 203] but other studies have shown that NO stimulates bone resorption.[204] The latter is crucial, as it raises the possibility that NO mediates articular erosions in RA and offers the therapeutic promise that NOS inhibitors might prevent joint damage in RA. NO is clearly also relevant to other conditions exhibiting excessive osteoclastic bone resorption, such as Paget's disease and osteoporosis.

INFECTION AND CANCER

Cytotoxicity against microbes and tumour cells was one of the first recognised and most studied actions of NO. NO mediates the non-specific component of the T cell immune response and is particularly targeted against intracellular microbes and protozoa. NO mediated microbial killing can occur independently of T cells, as shown by the resistance of immunodeficient mice to Listeria.[205] NO is also implicated in the response against or killing of leishmania, schistosoma, trypanosomes, *Plasmodium falciparum*, *Mycobacterium leprae* and *M tuberculosis*, *Legionella pneumophilia*, and several viruses.[206–212] The

latter exclude some of the most important bacterial pathogens that are extracellular targets of the oxygen dependent cytotoxic mechanisms of PMN, though PMN can utilise NO in killing *Staphylococcus aureus*.[213]

Candidate mechanisms by which NO effects cytostasis or cytotoxicity are via inhibition of iron centred enzymes and the formation of peroxynitrite, respectively. The former might explain the long recognised positive association between iron and infection. Indeed, excess iron reverses the cytostatic effects of NO on *Trypanosoma sp.*[214] However, another feature of trypanosomal infection, immunosuppression of the host, is also mediated by NO. In a manner analogous to the allogeneic mixed lymphocyte reaction, NO synthesis by host macrophages suppresses T cell proliferation.[215] Despite these findings, the evidence strongly favours a net antimicrobial effect for NO.

There are, however, circumstances in which NO may be detrimental. It has been hypothesised that excessive NO synthesis accounts for CNS dysfunction in cerebral malaria,[216] and there is definitive evidence that NO is the final common pathway leading to septic shock. The latter remains an important cause of mortality, especially in patients with underlying disease or the immunosuppressed—categories into which many rheumatic disease sufferers fall. Septic shock is initiated by endotoxins derived from bacterial cell walls which activate several humoral pathways and, most importantly, stimulate excessive cytokine release. Most interest has focused on TNFα as the principal mediator, though other cytokines are involved, as outcome correlates with the levels of IL-1, IFNγ and TNFα.[217] LPS, IL-1, IFNγ and TNFα are also the principal mediators of inducible NO synthesis in endothelial cells and VSMC, which are the probable cellular source of excessive intravascular NO release in septic shock.[188 189] In man, increased NO production occurs in sepsis and greater concentrations are associated with decreased systemic vascular resistance and greater circulating concentrations of endotoxin.[188] In animal models of septic shock, hypotension is reversed by NOS inhibitors;[218 219] however, high doses of the inhibitors may accelerate hypotension,[220] resulting in underperfusion of vital organs and other potentially detrimental effects. Initial reports were that LNMMA reversed hypotension[221 222] in patients with septic shock. A small randomised double blind placebo controlled study has shown that LNMMA increases blood pressure, systemic and pulmonary vascular resistance, and central venous pressure, but decreases cardiac output.[223] Further studies are clearly required to assess its possible adverse effects and confirm its efficacy and influence on outcome. Selective inhibition of the inducible NOS of endothelial cells and VSMC may improve the therapeutic benefit/risk equation and be more successful than inhibition of either endotoxin or TNFα.

Cytokine induced hypotension is also crucial in deploying immunotherapy against cancer. Hypotension is a serious complication of IL-2 treatment which, in clinical trials, has shown therapeutic benefit to patients with advanced melanoma and renal cell carcinoma.[224] Two separate studies provided evidence that NO synthesis is dramatically increased after IL-2 treatment[190 191] and that NO_2^-/NO_3^- concentrations correlate inversely with mean blood pressure.[192] If planned clinical trials of NOS inhibitors prove successful, it may be possible to use larger doses of IL-2, with the prospect of improved clinical response.[225]

The tumouricidal effects of macrophage NO production are well recognised and amongst several candidate mechanisms is the inhibition of ribonucleotide reductase, probably via an essential tyrosyl radical.[226] Moreover, conversion of L-arginine to ornithine/urea via arginase, and to NO via NOS are associated with tumour progression and rejection, respectively.[227] Antitumour treatment strategies involving NO include stimulating macrophage iNOS[228] and selectively impairing tumour blood flow with NOS inhibitors.[229] However, NO also has carcinogenic potential, as it alters DNA in vitro and is mutagenic to bacteria[230] and human cell lines;[231] furthermore, NO derived nitrosamines could explain the association between malignancy and inflammation. These effects are also highly relevant to inflammation, as NO attacks pancreatic islet cell DNA,[232] impairs enzymes that repair DNA,[233] produces a pattern of DNA fragmentation typical of apoptosis,[234] and directly induces apoptosis in tumour cells[235] and macrophages.[234] Recent evidence implicates p53, the tumour suppressor gene, in NO induced apoptosis, as NO and NO inhibitors respectively stimulate and prevent p53 expression and apoptosis.[236]

Other actions of NO

NO regulates basal pulmonary vascular tone,[237] mediates vasodilatation, and attenuates vasoconstriction in vitro[238] and ex vivo.[239] Moreover, NO mediated responses are impaired in animals[240] and humans with chronic hypoxia.[241–243] Because of its local delivery and breakdown, inhaled NO is a *selective* pulmonary vasodilator that reverses acute hypoxic pulmonary vasoconstriction in vivo.[237] In human studies, inhaled NO was beneficial in persistent pulmonary hypertension of the newborn[244 245] and adult respiratory distress syndrome.[246] NO mediated NANC nerve bronchodilatation is also the sole neural mechanism of airways dilatation in man.[135] Inhaled NO decreases methacholine induced bronchoconstriction in animals,[136] probably by directly relaxing bronchial smooth muscle, and has recently been shown to have a similar effect in humans.[247] NO can also mediate neurogenic inflammation in the lung,[178] though histamine modulates mast cell degranulation by a negative feedback loop involving NO activation of GCase.[248] Intriguingly, NO is also involved in the upregulation of ciliary beating,[249] which is inhibited by pyocyanin from *Pseudomonas aeruginosa*.[250]

NO has been investigated most extensively in the gut, in respect of its role in NANC

neurotransmission. NO has been directly implicated in the control of sphincters of the lower oesophagus,[137] ileocolonic junction,[131] and pylorus.[251] The last of these is of practical importance in infantile hypertrophic pyloric stenosis, in which defective pyloric relaxation may be aetiological. The absence of NADPH diaphorase activity in the hypertrophied circular musculature of affected infants suggests that a lack of NO synthase is the cause of pylorospasm, and perhaps the primary cause of this disorder;[252] this concept is supported by similar abnormalities in a mouse nNOS gene knockout model.[138] Increased NO synthesis is implicated in the haemodynamic consequences of cirrhosis leading to the hepatorenal syndrome.[253] It contrasts with the contribution of decreased blood flow to acute non-steroidal gastric lesions in rats.[254] NOS inhibitors reduce gastric mucosal blood flow[255] and attenuate increased blood flow associated with gastric acid secretion,[256] whilst organic nitrates reduce ethanol induced mucosal damage,[257] but NOS inhibitors only cause mucosal damage in the presence of other factors such as the inhibition of vasodilatory prostaglandins and neuropeptides.[258] Whilst animal studies suggest NO mediates the gastroprotective effects of several agents, NO is also implicated in gut inflammation—a paradox explained by the existence of constitutive and cytokine induced NOS. Increased NOS activity has been demonstrated in animal models of both ileal[259] and colonic inflammation,[260] whilst NOS inhibitors alleviate inflammation in a guinea pig model of ileitis.[261] In man there is both indirect[192] and direct evidence[193] of increased NOS activity in ulcerative colitis, though not Crohn's disease.

The widespread actions of this simple organic molecule are astonishing, and clearly relevant to pathophysiological events in many tissues, circumstances and diverse medical fields. NO has many actions relevant to rheumatic diseases, being implicated in inflammation and immunoregulation, hypoxic-reperfusion injury and vasculitis, cartilage and bone physiology, peptic ulceration, and pain mechanisms. Understanding the role of NO in a wider context should also help clarify the influence of NO on rheumatic diseases. Studies are in progress in several clinical areas to establish the benefits and problems of manipulating NO synthesis and, together with current molecular studies of NOS expression and regulation, should improve the understanding of the multifarious roles of NO.

1 Palmer R M J, Ferrige A G, Moncada S. Nitric oxide accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; **327:** 524–6.

2 Palmer R M J, Ashton D S, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 1988; **333:** 664–6.

3 Palacios M, Knowles R G, Palmer R M J, Moncada S. Nitric oxide from L-arginine stimulates the soluble guanylate cyclase in adrenal glands. *Biochem Biophys Res Commun* 1989; **165:** 802–9.

4 Radomski M W, Palmer R M, Moncada S. An L-arginine/nitric oxide pathway present in human platelets regulates aggregation. *Proc Natl Acad Sci USA* 1990; **87:** 5193–7.

5 Werner-Felmayer G, Werner E R, Fuchs D, Hausen A, Reibnegger G, Wachter H. Tetrahydrobiopterin-dependent formation of nitrite and nitrate in murine fibroblasts. *J Exp Med* 1990; **172:** 1599–607.

6 Yui Y, Hattori R, Kosuga K, *et al.* Calmodulin-independent nitric oxide synthase from rat polymorphonuclear neutrophils. *J Biol Chem* 1991; **266:** 3369–71.

7 Knowles R G, Palacios M, Palmer R M J, Moncada S. Formation of nitric oxide from L-arginine in the central nervous system: A transduction mechanism for stimulation of the soluble guanylate cyclase. *Proc Natl Acad Sci USA* 1989; **86:** 5159–62.

8 Bredt S D, Snyder S H. Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum. *Proc Natl Acad Sci USA* 1989; **86:** 9030–3.

9 Venturini C M, Knowles R G, Palmer R M J, Moncada S. Synthesis of nitric oxide in the bovine retina. *Biochem Biophys Res Commun* 1991; **180:** 920–5.

10 Li C G, Rand M J. Evidence for a role of nitric oxide in the neurotransmitter system mediating relaxation of the rat anococcygeus muscle. *Clin Exp Pharmacol Physiol* 1989; **16:** 933–8.

11 Radomski M W, Palmer R M J, Moncada S. Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proc Natl Acad Sci USA* 1990; **87:** 10043–7.

12 Kilbourn R G, Belloni P. Endothelial cell production of nitrogen oxides in response to interferon gamma in combination with tumour necrosis factor, interleukin-1 or endotoxin. *J Natl Cancer Inst* 1990; **82:** 772–6.

13 Beasley D, Schwartz J H, Brenner B M. Interleukin-1 induces prolonged L-arginine-dependent cyclic guanosine monophosphate and nitrite production in rat vascular smooth muscle cells. *J Clin Invest* 1991; **87:** 602–8.

14 Hibbs J B, Vavrin Z, Taintor R R. L-Arginine is required for the expression of the activated macrophage effector mechanism causing selective metabolic inhibition in target cells. *J Immunol* 1987; **138:** 550–65.

15 Stuehr D J, Nathan C F. Nitric oxide. A macrophage product responsible for cytostasis and respiratory inhibition in tumour target cells. *J Exp Med* 1989; **169:** 1543–55.

16 McCall T B, Boughton-Smith N K, Palmer R M J, Whittle B J R, Moncada S. Synthesis of nitric oxide from L-arginine by neutrophils. *Biochem J* 1989; **261:** 293–6.

17 Kirk S J, Regan M C, Barbul A. Cloned murine T lymphocytes synthesize a molecule with the biological characteristics of nitric oxide. *Biochem Biophys Res Commun* 1990; **173:** 660–5.

18 Curran R D, Billiar T R, Stuehr D J, Hofmann K, Simmons R L. Hepatocytes produce nitrogen oxides from L-arginine in response to inflammatory stimuli. *J Exp Med* 1989; **170:** 1769–74.

19 Salvemini D, Masini E, Anggard E, Mannaioni P F, Vane J. Synthesis of a nitric oxide-like factor from L-arginine by rat serosal mast cells: stimulation of guanylate cyclase and inhibition of platelet aggregation. *Biochem Biophys Res Commun* 1990; **169:** 596–601.

20 Marsden P A, Ballermann B J. Tumour necrosis factor α activates soluble guanylate cyclase in bovine glomerular mesangial cells via an L-arginine-dependent mechanism. *J Exp Med* 1990; **172:** 1843–52.

21 Stadler J, Stefanovic-Racic M, Billiar T R, *et al.* Articular chondrocytes synthesize nitric oxide in response to cytokines and lipopolysaccharide. *J Immunol* 1991; **147:** 3915–20.

22 Palmer R M J, Andrews T, Foxwell N A, Moncada S. Glucocorticoids do not affect the induction of a novel calcium dependent nitric oxide synthase in rabbit chondrocytes. *Biochem Biophy Res Commun* 1992; **188:** 209–15.

23 Stefanovic-Racic M, Stadler J, Georgescu H I, Evans C H. Nitric oxide production by cytokine stimulated synovial fibroblasts [abstract]. *Trans Orthop Res Soc* 1992; **17:** 228.

24 Bredt S D, Snyder S H. Isolation of nitric oxide synthase a calmodulin-requiring enzyme. *Proc Natl Acad Sci USA* 1990; **87:** 683–5.

25 Mayer B, John M, Bohme E. Purification of a Ca^{2+}/calmodulin-dependent nitric oxide synthase from porcine cerebellum. *FEBS Lett* 1990; **277:** 215–9.

26 Bredt S D, Hwang P M, Glatt C E, Lowenstein C, Reed R R, Snyder S H. Cloned and expressed nitric oxide structurally resembles P-450 reductase. *Nature* 1991; **351:** 714–8.

27 Nishida K, Harrison D G, Navas J P, *et al.* Molecular cloning and characterization of constitutive bovine aortic endothelial cell nitric oxide synthase. *J Clin Invest* 1992; **90:** 2092–6.

28 Sessa W C, Harrison J K, Barber C M, *et al.* Molecular cloning and expression of a cDNA encoding endothelial cell nitric oxide synthase. *J Biol Chem* 1992; **267:** 15274–6.

29 Lamas S, Marsden P A, Li G K, Tempst P, Michel T. Endothelial nitric oxide synthase: molecular cloning and characterization expression of a distinctive constitutive isoform. *Proc Natl Acad Sci USA* 1992;**89:**6348–52.

30 Janssens S P, Shimouchi A, Quertermous T, Bloch D B, Bloch K D. Cloning and expression of a cDNA encoding human endothelium derived relaxing factor/nitric oxide synthase. *J Biol Chem* 1992; **267:** 14519–22.

31 Marsden P A, Schappert K T, Chen H S, *et al.* Molecular cloning and characterization of human endothelial nitric oxide synthase. *FEBS Lett* 1992; **307:** 287–93.

32 Xie Q W, Cho H J, Calaycay J, *et al.* Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. *Science* 1992; **256:** 225–8.

33 Stuehr D J, Cho H J, Kwon N S, Weisse M F, Nathan C F. Purification and characterization of the cytokine induced macrophage nitric oxide synthesis: an FAD and FMN containing flavoprotein. *Proc Natl Acad Sci USA* 1991; **88:** 7773–7.

34 Yui Y, Hattori R, Kosuga K, Eizawa H, Hiki K, Kawai C. Purification of nitric oxide synthase from rat macrophages. *J Biol Chem* 1991; **266:** 12544–7.

35 Hevel J M, White K A, Marletta M A. Purification of the inducible murine macrophage nitric oxide synthase. *J Biol Chem* 1991; **266:** 22789–91.

36 Lyons C L, Orloff G J, Cunningham J M. Molecular cloning and functional expression of an inducible nitric oxide synthase from a murine macrophage cell line. *J Biol Chem* 1992; **267:** 6370–74.

37 Marsden P A, Heng H H Q, Scherer S W, *et al.* Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. *J Biol Chem* 1993; **268:** 17478–88.

38 Spurr N, Liao M, Lizhi L, Emmson P, Weiming X. Localization of brain nitric oxide synthase to human chromosome 12. *Genomics* 1992; **14:** 802–4.

39 Chartrain N A, Geller D A, Koty P P, *et al.* Molecular cloning, structure and chromosomal localization of the human inducible nitric oxide synthase gene. *J Biol Chem* 1994; **269:** 6765–72.

40 Marsden P A, Heng H H Q, Duff C, Shi X M, Tsui L C, Hall A V. Localization of the human gene for inducible nitric oxide synthase to chromosome 17q11.2-q12. *Genomics* 1994; **19:** 183–5.

41 White K A, Marletta M A. Nitric oxide synthase is a cytochrome P-450 type hemoprotein. *Biochemistry* 1992; **31:** 6627–31.

42 Cho H J, Xie Q W, Calaycay J, *et al.* Calmodulin is a subunit of nitric oxide synthase from macrophages. *J Exp Med* 1992; **176:** 599–604.

43 Bredt S D, Ferris C D, Snyder S H. Nitric oxide synthase regulatory sites. Phosphorylation by cyclic AMP dependent protein kinase, protein kinase C and calcium/calmodulin protein kinase: identification of flavin and calmodulin binding sites. *J Biol Chem* 1992; **267:** 10976–81.

44 Cunha F G, Moncada S, Liew F Y. Interleukin 10 inhibits the induction of nitric oxide synthase by interferon gamma in murine macrophages. *Biochem Biophys Res Commun* 1992; **182:** 1155–9.

45 Nelson B J, Ralph P, Green S J, Nacy C A. Differential susceptibility of activated macrophage cytotoxic effector reactions to the suppressive effects of transforming growth factor β. *J Immunol* 1991; **146:** 1849–57.

46 Al-Ramadi B K, Meissler J J, Huang D, Eisenstein T K. Immunosuppression induced by nitric oxide and its inhibition by interleukin-4. *Eur J Immunol* 1992; **22:** 2249–54.

47 Oswald I P, Gazzinelli R T, Sher A, James S L. IL-10 synergizes with IL-4 and transforming growth factor-β to inhibit macrophage cytotoxic activity. *J Immunol* 1992; **148:** 3578–82.

48 McCall, T B, Palmer R M J, Moncada S. Interleukin-8 inhibits the induction of nitric oxide synthase in rat peritoneal neutrophils. *Biochem Biophys Res Commun* 1991; **186:** 680–5.

49 Lorsbach R B, Russell S W. A specific sequence of stimulation is required to induce synthesis of the antimicrobial molecule nitric oxide by mouse macrophages. *Infect Immun* 1992; **60:** 2133–5.

50 Bogdan C, Vodovotz Y, Paik J, Xie Q W, Nathan C. Traces of bacterial lipopolysaccharide suppress IFN-gamma-induced nitric oxide synthase gene expression in primary mouse macrophages. *J Immunol* 1993; **151:** 301–9.

51 Lorsbach R B, Murphy W J, Lowenstein C J, Snyder S H, Russell S W. Expression of the nitric oxide synthase gene in mouse macrophages activated for tumor cell killing. Molecular basis for the synergy between interferon-gamma and lipopolysaccharide. *J Biol Chem* 1993; **268:** 1908–13.

52 Xie Q W, Whinant R, Nathan C. Promoter of the mouse gene encoding calcium-independent nitric oxide synthase confers inducibility by interferon γ and bacterial lipopolysaccharide. *J Exp Med* 1993; **177:** 1779–84.

53 Martin E, Nathan C, Xie Q W. Role of interferon regulatory factor 1 in induction of nitric oxide synthase. *J Exp Med* 1994; **180:** 977–84.

54 Drapier J C, Hibbs J B. Differentiation of murine macrophages to express non-specific cytotoxicity for tumor cells results in L-arginine dependent inhibition of mitochondrial iron-sulfur enzymes in the macrophage effector cells. *J Immunol* 1988; **140:** 2829–38.

55 Dimmeler S, Lottspeich F, Brune B. Nitric oxide causes ADP-ribosylation and inhibition of glyceraldehyde-3-phosphate dehydrogenase. *J Biol Chem* 1992; **267:** 16771–4.

56 Hibbs J B, Taintor R R, Vavrin Z. Iron depletion possible cause of tumor cytotoxicity induced by activated macrophages. *Biochem Biophys Res Commun* 1984; **123:** 716–23.

57 Reif D W, Simmons R D. Nitric oxide mediates iron release from ferritin. *Arch Biochem Biophys* 1990; **283:** 537–41.

58 Weiss G, Goossen B, Doppler W, *et al.* Translational regulation via iron-responsive elements by the nitric oxide/NO-synthase pathway. *EMBO J* 1993; **12:** 3651–7.

59 Drapier J C, Hirling H, Wietzerbin J, Kaldy P, Kuhn L C. Biosynthesis of nitric oxide activates iron regulatory factor in macrophages. *EMBO J* 1993; **12:** 3643–9.

60 Gryglewski R J, Palmer R M J, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived relaxing factor. *Nature* 1986; **320:** 454–6.

61 Zweier J L. Measurement of superoxide derived free radicals in the reperfused heart: evidence for a free radical mechanism of reperfusion injury. *J Biol Chem* 1988; **263:** 1353–7.

62 Farrell A J, Williams R B, Stevens C R, Lawrie A S, Cox N L, Blake D R. Exercise induced release of von Willebrand factor: evidence for hypoxic microvascular injury in rheumatoid arthritis. *Ann Rheum Dis* 1992; **51:** 1117–22.

63 Beckman J S, Beckman T W, Chen J, Marshall P A, Freeman B A. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci USA* 1990; **87:** 1620–24.

64 Yang G, Candy T, Boaro M, *et al.* Free radical yields from the homolysis of peroxynitrous acid. *Free Radic Biol Med* 1992; **12:** 327–30.

65 Kelm M, Schrader J. Control of coronary vascular tone by nitric oxide. *Circ Res* 1990; **66:** 1561–75.

66 Keaney J F, Simon D I, Stamler J S, *et al.* NO forms an adduct with serum albumin that has endothelium-derived relaxant factor like properties. *J Clin Invest* 1993; **91:** 1582–9.

67 Amezcua J L, Palmer R M J, de Souza B M, Moncada S. Nitric oxide synthesized from L-arginine regulates vascular tone in the coronary circulation of the rabbit. *Br J Pharmacol* 1989; **97:** 1119–24.

68 Chu A, Chambers D E, Lin C C, *et al.* Effects of inhibition of nitric oxide formation on basal vasomotion and endothelium-dependent responses of the coronary arteries in awake dogs. *J Clin Invest* 1991; **87:** 1964–8.

69 Ekelund U, Mellander S. Role of endothelium-derived nitric oxide in the regulation of tonus in large-bore arterial resistance vessels, arterioles and veins in cat skeletal muscle. *Acta Physiol Scand* 1990; **140:** 301–9.

70 Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* 1989; **334:** 997–1000.

71 Vallance P, Collier J, Moncada S. Nitric oxide synthesised from L-arginine mediates endothelium dependent dilatation in human veins in vivo. *Cardiovasc Res* 1989; **23:** 1053–7.

72 Werns S W, Walton J A, Hsai H H, Nabel E G, Sanz M L, Pitt B. Evidence of endothelial dysfunction in angiographically normal coronary arteries with coronary artery disease. *Circulation* 1989; **79:** 287–91.

73 Chester A H, O'Neil G S, Moncada S, Tadjkarimi S, Yacoub M H. Low basal and stimulated release of nitric oxide in atherosclerotic epicardial coronary arteries. *Lancet* 1990; **336:** 897–900.

74 Andrews H E, Bruckdorfer K R, Dunn R C, Jacobs M. Low-density lipoproteins inhibit endothelium dependent relaxation in rabbit aorta. *Lancet* 1987; **327:** 237–9.

75 Girerd X J, Hirsch A T, Cooke J P, Dzau V J, Creager M A. L-Arginine restores cholinergic relaxation of hypercholesterolaemic rabbit thoracic aorta. *Circ Res* 1990; **67:** 1301–8.

76 Drexler H, Zeiher A M, Meinzer K, Just H. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolaemic patients by L-arginine. *Lancet* 1991; **338:** 1546–50.

77 Jacobs M, Plane F, Bruckdorfer K R. Native and oxidized low-density lipoproteins have different inhibitory effects on endothelium-derived relaxing factor in the rabbit aorta. *Br J Pharmacol* 1990; **100:** 21–6.

78 Schmidt K, Graier W F, Kostner G M, Mayer B, Kukovetz W R. Activation of soluble guanylate cyclase by nitrovasodilators is inhibited by oxidized low-density lipoprotein. *Biochem Biophys Res Commun* 1990; **172:** 614–9.

79 Yates M T, Lambert L E, Whitten J P, *et al.* A protective role for nitric oxide in the oxidative modification of low density lipoproteins by mouse macrophages. *FEBS Lett* 1992; **309:** 135–8.

80 Steinberg D, Parthasarathy S, Carew T E, Khoo J C, Witztum J L. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989; **320:** 915–24.

81 Matheis G, Sherman M P, Buckberg G D, Haybron D M, Young H H, Ignarro L J. Role of L-arginine-nitric oxide pathway in myocardial reoxygenation injury. *Am J Physiol* 1992; **262:** H616–20.

82 Buisson A, Plotkine M, Boulu R G. The neuroprotective effect of a nitric oxide inhibitor in a rat model of focal cerebral ischaemia. *Br J Pharmacol* 1992; **106:** 766–7.

83 Radomski M W, Palmer R M J, Moncada S. Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. *Lancet* 1987; **2:** 1057–8.

84 Yao S K, Ober J C, Krishnaswami A, *et al.* Endogenous nitric oxide protects against platelet aggregation and cyclic flow variations in stenosed and endothelium-injured arteries. *Circulation* 1992; **86:** 1302–9.

85 Radomski M W, Palmer R M J, Moncada S. The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *Br J Pharmacol* 1987; **92:** 639–46.

86 Garg U C, Hassid A. Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest* 1989; **83:** 1774–7.

87 Braughler J M. Soluble guanylate cyclase activation by nitric oxide and its reversal. Involvement of sulfhydryl group oxidation and reduction. *Biochem Pharmacol* 1983; **32:** 811–8.

88 Axelsson K L, Andersson R G. Tolerance towards nitroglycerin, induced in vivo, is correlated to a reduced cGMP response and an alteration in cGMP turnover. *Eur J Pharmacol* 1983; **88:** 71–9.

89 Calver A, Collier J, Moncada S, Vallance P. Effect of local intra-arterial NG-monomethyl-L-arginine in patients with hypertension: the nitric oxide dilator mechanism appears abnormal. *J Hypertens* 1992; **10:** 1025–31.

90 Danaberg J, Sider R S, Grekin R J. Sustained hypertension induced by orally administered nitro-L-arginine. *Hypertension* 1993; **21:** 359–63.

91 Baylis C, Mitruka B, Deng A. Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. *J Clin Invest* 1992; **90:** 278–81.

92 Sakuma I, Togashi H, Yoshioka M, *et al.* NG-methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo. A role for nitric oxide in the central regulation of sympathetic tone? *Circ Res* 1992; **70:** 607–11.

93 Lahera V, Salom M G, Miranda-Guardiola F, Moncada S, Romero J C. Effects of NG-nitro-L-arginine methyl ester on renal function and blood pressure. *Am J Physiol* 1991; **261:** F1033–7.

94 Scholz H, Kurtz A. Involvement of NO in the pressure control of renin secretion from isolated perfused kidney. *J Clin Invest* 1993; **91:** 1088–94.

95 Lahera V, Salazar J, Salom M G, Romero J C. Deficient production of nitric oxide induces volume-dependent hypertension. *J Hypertens* 1992; **10:** S173–7.

96 Chen P Y, Sanders P W. L-Arginine abrogates salt sensitive hypertension in Dahl/Rapp rats. *J Clin Invest* 1991; **88:** 1559–67.

97 Boje K M, Arora P K. Microglial produced nitric oxide and reactive nitrogen oxides mediate neuronal cell death. *Brain Res* 1992; **587:** 250–6.

98 Bredt S D, Hwang P M, Snyder S H. Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature* 1990; **347:** 768–70.

99 Hope B T, Michael G J, Knigge K M, Vincent S R. Neuronal NADPH diaphorase is a nitric oxide synthase. *Proc Natl Acad Sci USA* 1991; **88:** 2811–14.

100 Dawson T M, Bredt D S, Fotuhi M, Hwang P M, Snyder S H. Nitric oxide synthase and neuronal NADPH diaphorase are identical in brain and peripheral tissues. *Proc Natl Acad Sci USA* 1991; **88:** 7797–801.

101 Ferrante R J, Kowall N W, Beal M F, Richardson E P, Bird E D, Martin J B. Selective sparing of a class of striatal neurons in Huntington's disease. *Science* 1985; **230:** 561–3.

102 Uemura Y, Kowall N W, Beal M F. Selective sparing of NADPH-diaphorase-somatostatin-neuropeptide Y neurons in ischaemic gerbil striatum. *Ann Neurol* 1990; **27:** 620–5.

103 Koh J Y, Choi D W. Vulnerability of cultured cortical neurons to damage by excitotoxins: differential susceptibility of neurons containing NADPH diaphorase. *J Neurosci* 1988; **8:** 2153–63.

104 Dawson V L, Dawson T M, London E D, Bredt D S, Snyder S H. Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proc Natl Acad Sci USA* 1991; **88:** 6368–71.

105 Nowicki J P, Duval D, Poignet H, Scatton B. Nitric oxide mediates neuronal death after focal cerebral ischaemia in the mouse. *Eur J Pharmacol* 1991; **204:** 339–40.

106 Trifiletti R R. Neuroprotective effects of NG-nitro-L-arginine in focal stroke in the 7 day old rat. *Eur J Pharmacol* 1992; **218:** 197–8.

107 Lipton S A, Choi Y B, Pan Z H, *et al.* A redox based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. *Nature* 1993; **364:** 626–32.

108 Lei S Z, Pan Z H, Aggarwal S R, *et al.* Effect of nitric oxide production on the redox modulatory site of the NMDA receptor channel complex. *Neuron* 1992; **8:** 1087–99.

109 Kollegger H, McBean G J, Tipton K F. Reduction of striatal *N*-methyl-D-aspartate toxicity by inhibition of nitric oxide synthase. *Biochem Pharmacol* 1993; **45:** 260–4.

110 Garthwaite J, Garthwaite G J. Cellular origins of cyclic GMP responses to excitatory amino acid receptor agonists in rat cerebellum in vitro. *J Neurochem* 1987; **48:** 29–39.

111 de Vente J, Bol J, Steinbusch H M W. Localization of cGMP in the cerebellum of the adult rat: an immunohistochemical study. *Brain Res* 1989; **504:** 332–7.

112 O'Dell T J, Hawkins R D, Kandl E R, Arancio O. Tests of the roles of two diffusible substances in long term potentiation: Evidence for nitric oxide as a possible early retrograde messenger. *Proc Natl Acad Sci USA* 1991; **88:** 11285–9.

113 Gally J A, Montague P R, Reeke G N, Edelman G M. The NO hypothesis: possible effects of a short lived, rapidly diffusible signal in the development and function of the nervous system. *Proc Natl Acad Sci USA* 1990; **87:** 3547–51.

114 Shibuki K, Okada D. Endogenous nitric oxide release required for long term synaptic depression in the cerebellum. *Nature* 1991; **349:** 326–8.

115 Izumi Y, Clifford D B, Zorumski C F. Inhibition of long term potentiation by NMDA-mediated nitric oxide release. *Science* 1992; **257:** 1273–6.

116 Chapman P F, Atkins C M, Allen M Y, Steinmetz J E. Inhibition of NO synthesis impairs two forms of learning. *Neuroreport* 1992; **3:** 567–70.

117 Faraci F M, Breese K R. Nitric oxide mediates vasodilatation in response to activation of *N*-methyl-D-aspartate receptor in brain. *Circ Res* 1993; **72:** 476–80.

118 Northington F J, Matherene G P, Berne R M. Competitive inhibition of NO synthase prevents the cortical hyperaemia associated with peripheral nerve stimulation. *Proc Natl Acad Sci USA* 1992; **89:** 6649–52.

119 Dirnagl U, Lindauer U, Villringer A. Role of nitric oxide in the coupling of cerebral blood flow to neuronal activation in rats. *Neurosci Lett* 1993; **149:** 43–6.

120 Duarte I D G, Lorenzetti B B, Ferreira S H. Peripheral analgesia and activation of the nitric oxide-cyclic GMP pathway. *Eur J Pharmacol* 1990; **186:** 289–93.

121 Duarte I D G, dos Santos I R, Lorenzetti B B, Ferreira S H. Analgesia by direct antagonism of nociceptor sensitization involves the arginine-nitric oxide-cyclic GMP pathway. *Eur J Pharmacol* 1992; **217:** 225–7.

122 Kawabata A, Fukuzumi Y, Fukushima Y, Takagi H. Antinociceptive effect of L-arginine on the carrageenin induced hyperalgesia of the rat: possible involvement of central opioidergic systems. *Eur J Pharmacol* 1992; **218:** 153–8.

123 Kawabata A, Nishimura Y, Takagi H. L-Leucyl-L-arginine, naltrindole and D-arginine block antinociception elicited by L-arginine in mice with carrageenin induced hyperalgesia. *Br J Pharmacol* 1992; **107:** 1096–101.

124 Tseng L F, Xu J Y, Pieper G M. Increase of nitric oxide production by L-arginine potentiates i.c.v. administered beta-endorphin induced antinociception in the mouse. *Eur J Pharmacol* 1992; **212:** 301–3.

125 Moore P K, Oluyomi A O, Babbedge R C, Wallace P, Hart S L. L-N^G-nitroarginine methyl ester exhibits antinociceptive activity in the mouse. *Br J Pharmacol* 1991; **102:** 198–202.

126 Haley J A, Dickenson A H, Schachter M. Electrophysiological evidence of a role for nitric oxide in prolonged chemical nociception in the rat. *Neuropharmacol* 1992; **31:** 251–8.

127 Meller S T, Pechman P S, Gebhart G F, Maves T J. Nitric oxide mediates the thermal hyperalgesia produced in a model of neuropathic pain in the rat. *J Neurosci* 1992; **50:** 7–10.

128 Kolesnikov Y A, Pick C G, Pasternak G W. N^G-Nitro-L-arginine prevents morphine tolerance. *Eur J Pharmacol* 1992; **221:** 399–400.

129 Pape H C, Mager R. Nitric oxide controls oscillatory activity in thalamocortical neurons. *Neuron* 1992; **9:** 441–8.

130 Gillespie J S, Liu X, Martin W. The effect of L-arginine and N^G-monomethyl-L-arginine on the response of the rat anococcygeus to NANC nerve stimulation. *Br J Pharmacol* 1989; **98:** 1080–2.

131 Boeckxstaens G E, Pelckmans P A, Bult H, De Man J G, Herman A G, Van Maercke Y M. Non-adrenergic non-cholinergic relaxation mediated by nitric oxide in the canine ileocolonic junction. *Eur J Pharmacol* 1990; **190:** 239–46.

132 Ignarro L J, Bush P A, Buga G M, Wood K S, Fukuto J M, Rajfer J. Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. *Biochem Biophys Res Commun* 1990; **170:** 843–50.

133 Belvisi M G, Stretton D, Barnes P J. Nitric oxide as an endogenous modulator of cholinergic neurotransmission in guinea-pig airways. *Eur J Pharmacol* 1991; **198:** 219–21.

134 Toda N, Okamura T. L-N^G-monomethyl-L-arginine of the response to nerve stimulation in isolated dog mesenteric and cerebral arteries. *Jap J Pharmacol* 1990; **52:** 170–3.

135 Burnett A L, Lowenstein C J, Bredt D S, Chang T S K, Snyder S H. Nitric oxide a physiological mediator of penile erection. *Science* 1992; **257:** 401–3.

136 Dupuy P M, Shore S A, Drazen J M, Frostell C, Hill W A, Zapol W M. Bronchodilator action of inhaled nitric oxide in guinea pigs. *J Clin Invest* 1992;**90:**421–8.

137 Tottrup A, Svane D, Forman A. Nitric oxide mediating NANC inhibition in opossum lower esophageal sphincter. *Am J Physiol* 1991; **260:** G385–9.

138 Hwang P M, Dawson T M, Bredt S D, Snyder S H, Fishman M C. Targeted disruption of the neuronal nitric oxide synthase gene. *Cell* 1993; **75:** 1273–86.

139 Kulka J P. The pathogenesis of rheumatoid arthritis. *J Chron Dis* 1959; **10:** 388–402.

140 Wakefield A J, Sawyer A M, Dhillon A P, *et al.* Pathogenesis of Crohn's disease: multifocal gastrointestinal infarction. *Lancet* 1989; **2:** 1057–62.

141 Blake D R, Merry P, Unsworth J, *et al.* Hypoxic-reperfusion injury in the inflamed human joint. *Lancet* 1989; **1:** 289–93.

142 Freay A D, Johns A, van Breemen C, Rubanyi G M. Pressure depresses the release of endothelium-derived relaxing factor from isolated canine arteries. In: Rubanyi G M, Vanhoutte P M, eds. *Endothelium-derived contracting factors*. Basel: Karger, 1990.

142a Granger D N, Benoit J N, Suzuki M, Grisham M B. Leukocyte adherence to venular endothelium during ischaemia-reperfusion. *Am J Physiol* 1989; **257:** G683–8.

143 Xin L M, Weyrich A S, Lefer D J, Lefer A M. Diminished basal nitric oxide release after myocardial ischaemia-reperfusion and reperfusion promotes neutrophil adherence to coronary endothelium. *Circ Res* 1993; **72:** 403–12.

144 Kubes P, Suzuki M, Granger D N. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci USA* 1991; **88:** 4651–5.

145 Kurose I, Kubes P, Wolf R, *et al.* Inhibition of nitric oxide production. Mechanisms of vascular albumin leakage. *Circ Res* 1993; **73:** 164–71.

146 Kubes P, Kanwar S, Niu X F, Gaboury J P. Nitric oxide synthesis inhibition induces leukocyte adhesion via superoxide and mast cells. *FASEB J* 1993; **7:** 1293–9.

147 Hogaboam C M, Befus A D, Wallace J L. Modulation of rat mast cell reactivity by IL-1 beta. Divergent effects on nitric oxide and platelet-activating factor release. *J Immunol* 1993; **151:** 3767–74.

148 Salvemini D, Masini E, Pistelli A, Mannaioni P F, Vane J. Nitric oxide: a regulatory mediator of mast cells reactivity. *J Cardiovasc Pharmacol* 1991; **17** (suppl 3): S258–64.

149 Mulligan M S, Hevel J M, Marletta M A, Ward P A. Tissue injury caused by deposition of immune complexes is L-arginine dependent. *Proc Natl Acad Sci USA* 1991; **88:** 6338–42.

150 Mulligan M S, Warren J S, Smith C W, *et al.* Lung injury after deposition of IgA immune complexes. Requirements for CD18 and L-arginine. *J Immunol* 1992; **148:** 3086–92.

151 Kaplan S S, Billar T R, Curran R D, Zdziarski U E, Simmons R L, Basford R E. Inhibition of chemotaxis with *N*-monomethyl-L-arginine: a role for cGMP. *Blood* 1989; **74:** 1885–7.

152 Bath P M W, Hassall D G, Gladwin A M, Palmer R M J, Martin J F. Nitric oxide and prostacyclin. Divergence of inhibitory effects on monocyte chemotaxis and adhesion to endothelium in vitro. *Arterioscler Thromb* 1991; **11:** 254–60.

153 Carreras M C, Pargament G A, Catz S D, Poderoso J J, Boveris A. Kinetics of nitric oxide and hydrogen peroxide production and formation of peroxynitrite during the respiratory burst of human neutrophils. *FEBS Lett* 1994; **341:** 65–8.

154 Bryant J L, Mehta P, Van der Porten A, Mehta J L. Co-purification of 130 kD nitric oxide synthase and a 22 kD link protein from human neutrophils. *Biochem Biophys Res Commun* 1992; **189:** 558–64.

155 Clancy R M, Leszczynska-Piziak J, Abramson S B. Nitric oxide, an endothelial cell relaxation factor, inhibits neutrophil superoxide anion production via a direct action on the NADPH oxidase. *J Clin Invest* 1992; **90:** 1116–21.

156 Rinaldo J E, Clark M, Parinello J, Shepherd V L. Nitric oxide inactivates xanthine dehydrogenase and xanthine oxidase in interferon-gamma-stimulated macrophages. *Am J Respir Cell Mol Biol* 1994; **11:** 625–30.

157 Lander H M, Sehajpal P, Levine D M, Novogrodsky A. Activation of human peripheral blood mononuclear cells by nitric oxide-generating compounds. *J Immunol* 1993; **150:** 1509–16.

158 Efron D T, Kirk S J, Regan M C, Wasserkrug H, Barbul A. Nitric oxide generation from L-arginine is required for optimal human peripheral blood lymphocyte DNA synthesis. *Surgery* 1991; **110:** 327–34.

159 Barbul A, Lazarou S A, Efron D T, Wasserkrug H L, Efron G. Arginine enhances wound healing and lymphocyte immune responses in humans. *Surgery* 1990; **108:** 331–7.

160 Park K G M, Hayes P D, Garlick P J, Sewell H, Eremin O. Stimulation of lymphocyte natural cytotoxicity by L-arginine. *Lancet* 1991; **337:** 645–6.

161 Holt P G, Oliver J, Bilyk N, *et al.* Downregulation of the antigen presenting cell function(s) of pulmonary dendritic cells in vivo by resident alveolar macrophages. *J Exp Med* 1993; **177:** 397–407.

162 Hoffman R A, Langrehr J M, Billiar T R, Curran R D, Simmons R L. Alloantigen induced activation of rat splenocytes is regulated by the oxidative metabolism of L-arginine. *J Immunol* 1990; **145:** 2220–6.

163 Mills C D. Molecular basis of suppressor macrophages. Arginine metabolism via the nitric oxide synthetase pathway. *J Immunol* 1991; **146:** 2719–23.

164 Albina J E, Abate J A. Henry W L Jr. Nitric oxide production is required for murine resident peritoneal macrophages to suppress mitogen-stimulated T cell proliferation. Role of IFNγ in the induction of the nitric oxide-synthesizing pathway. *J Immunol* 1991; **147:** 144–8.

165 Langrehr J M, Hoffman R A, Billiar T R, Lee K K, Schraut W H, Simmons R L. Nitric oxide synthesis in the in vivo allograft response: a possible regulatory mechanism. *Surgery* 1991; **110:** 335–42.

166 Langrehr J M, Dull K E, Ochoa J B, *et al.* Evidence that nitric oxide production by in vivo allosensitized cells inhibits the development of allospecific CTL. *Transplantation* 1992; **53:** 632–40.

167 Wren S M, Hoffman R A, Billiar T R, Abou el Ezz A, Hronakes M L, Ildstad S T. T-cell mitogenic responses in fully xenogeneic chimeras (WF rat-B10 mouse) are restored by blocking the L-arginine-dependent nitric oxide pathway. *Transplant Proc* 1992; **24:** 499–500.

168 Lancaster J R Jr, Langrehr J M, Bergonia H A, Murase N, Simmons R L, Hoffman R A. EPR detection of heme and nonheme iron-containing protein nitrosylation by nitric oxide during rejection of rat heart allograft. *J Biol Chem* 1992; **267:** 10994–8.

169 Langrehr J M, Muller A R, Bergonia H A, *et al.* Detection of nitric oxide by electron paramagnetic resonance spectroscopy during rejection and graft-versus-host disease after small-bowel transplantation in the rat. *Surgery* 1992; **112:** 395–401.

170 Langrehr J M, Murase N, Markus P M, *et al.* Nitric oxide production in host-versus-graft and graft-versus-host reactions in the rat. *J Clin Invest* 1992; **90:** 679–83.

171 Garside P, Hutton A K, Severn A, Liew F Y, Mowat A M. Nitric oxide mediates intestinal pathology in graft-vs-host disease. *Eur J Immunol* 1992; **22:** 2141–5.

172 Taylor-Robinson A W, Liew F Y, Severn A, *et al.* Regulation of the immune response by nitric oxide differentially produced by T helper type 1 and T helper type 2 cells. *Eur J Immunol* 1994; **24:** 980–4.

173 Rengasamy A, Johns R A. Regulation of nitric oxide synthase by nitric oxide. *Mol Pharmacol* 1993; **44:** 124–8.

174 Ialenti A, Ianaro A, Moncada S, Di Rosa M. Modulation of acute inflammation by endogenous nitric oxide. *Eur J Pharmacol* 1992; **211:** 177–82.

175 Mayhan W G. Role of nitric oxide in modulating permeability of hamster cheek pouch in response to adenosine 5'-diphosphate and bradykinin. *Inflammation* 1992; **16:** 295–305.

176 Kuo H P, Liu S, Barnes P J. The effect of endogenous nitric oxide on neurogenic plasma exudation in guinea-pig airways. *Eur J Pharmacol* 1992; **221:** 385–8.

177 Hughes S R, Williams T J, Brain S D. Evidence that endogenous nitric oxide modulates oedema formation induced by substance P. *Eur J Pharmacol* 1990; **191:** 481–4.

178 Khalil Z, Helme R D. The quantitative contributin of nitric oxide and sensory nerves to bradykinin-induced inflammation in rat skin microvasculature. *Brain Res* 1992; **589:** 102–8.

179 Lippe I T, Stabentheiner A, Holzer P. Participation of nitric oxide in the mustard oil induced inflammation of the rat paw skin. *Eur J Pharmacol* 1993; **232:** 113–20.

180 Mandrup-Poulsen T, Helqvist S, Wogensen L D, *et al.* Cytokines and effector molecules in the destruction of pancreatic β cells. *Curr Top Microbiol Immunol* 1990; **164:** 169–93.

181 Corbett J A, Sweetland M A, Wang J L, Lancaster J R, McDaniel M L. Nitric oxide mediates cytokine induced inhibition of insulin secretion by human islets of Langerhans. *Proc Natl Acad Sci USA* 1993; **90:** 1731–5.

182 Southern C, Schulster D, Green I C. Inhibition of insulin secretion by interleukin-1 beta and tumour necrosis factor-alpha via an L-arginine-dependent nitric oxide generating mechanism. *FEBS Lett* 1990; **276:** 42–4.

183 Kroncke K D, Kolb-Bachofen V, Berschick B, Burkart V, Kolb H. Activated macrophages kill pancreatic syngeneic islet cells via arginine-dependent nitric oxide generation. *Biochem Biophys Res Comm* 1991; **175:** 752–8.

184 Burkart V, Imai Y, Kallmann B, Kolb H. Cyclosporin a protects pancreatic islet cells from nitric oxide-dependent macrophage cytotoxicity. *FEBS Lett* 1992; **313:** 56–8.

185 Kolb H, Kiesel U, Kroncke K D, Kolb-Bachofen V. Suppression of low dose streptozotocin induced diabetes in mice by administration of a nitric oxide synthase inhibitor. *Life Sci* 1991; **49:** PL213–7.

186 Corbett J A, Tilton R G, Chang K, *et al.* Aminoguanidine, a novel inhibitor of nitric oxide formation, prevents diabetic vascular dysfunction. *Diabetes* 1992; **41:** 552–6.

187 Farrell A J, Blake D R, Palmer R M, Moncada S. Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. *Ann Rheum Dis* 1992; **51:** 1219–22.

188 Ochoa J B, Udekwu A O, Billiar T R, *et al.* Nitrogen oxide levels in patients after trauma and during sepsis. *Ann Surg* 1991; **214:** 621–6.

189 Shi Y, Li H, Shen C, *et al.* Plasma nitric oxide levels in newborn infants with sepsis. *J Pediatr* 1993; **123:** 435–8.

190 Hibbs J B, Westenfelder C, Taintor R, *et al.* Evidence for cytokine inducible nitric oxide synthesis from L-arginine in patients receiving interleukin-2 therapy. *J Clin Invest* 1992; **89:** 867–77.

191 Ochoa J B, Curti B, Peitzman A B, *et al.* Increased circulating nitrogen oxides after human tumor immunotherapy: correlation with toxic hemodynamic changes. *J Natl Cancer Inst* 1992; **84:** 864–7.

192 Middleton S J, Shorthouse M, Hunter J O. Increased nitric oxide synthesis in ulcerative colitis. *Lancet* 1993; **341:** 465–6.

193 Boughton-Smith N K, Evans S M, Hawkey C J, *et al.* Nitric oxide synthase activity in ulcerative colitis and Crohn's disease. *Lancet* 1993; **342:** 338–40.

194 McCartney-Francis N, Allen J B, Mizel D E, *et al.* Suppression of arthritis by an inhibitor of nitric oxide synthase. *J Exp Med* 1993; **178:** 749–54.

195 Weinberg J B, Granger D L, Pisetsky D S, *et al.* The role of nitric oxide in the pathogenesis of spontaneous murine autoimmune disease: increased nitric oxide production and nitric oxide synthase expression in MRL-lpr/lpr mice, and reduction of spontaneous glomerulonephritis and arthritis by orally administered NG-monomethyl-L-arginine. *J Exp Med* 1994; **179:** 651–60.

196 Najafipour H, Ferrell W R. Nitric oxide modulates sympathetic vasoconstriction and basal blood flow in normal and acutely inflamed rabbit knee joints. *Exp Physiol* 1993; **78:** 615–24.

197 Zweier J L, Wang P, Samouliov A, Kuppusamy P. Enzyme-independent formation of nitric oxide in biological tissues. *Nature Medicine* 1995; **1:** 804–9.

198 Taskiran D, Stefanovic-Racic M, Georgescu H, Evans C. Nitric oxide mediates suppression of cartilage proteoglycan synthesis by interleukin-1. *Biochem Biophys Res Commun* 1994; **200:** 142–8.

199 Palmer R M J, Hickery M S, Charles I G, Moncada S, Bayliss M T. Induction of nitric oxide synthase in human chondrocytes. *Biochem Biophy Res Commun* 1993; **193:** 398–405.

200 Damoulis P D, Hauschka P V. Cytokines induce nitric oxide production in mouse osteoblasts. *Biochem Biophys Res Commun* 1994; **201:** 924–31.

201 Lowik C W, Nibbering P H, van de Ruit M, Papapoulos S E. Inducible production of nitric oxide in osteoblast-like cells and in fetal mouse bone explants is associated with suppression of osteoclastic bone resorption. *J Clin Invest* 1994; **93:** 1465–72.

202 Ralston S H, Todd D, Helfrich M, Benjamin N, Grabowski P S. Human osteoblast-like cells produce nitric oxide and express inducible nitric oxide synthase. *Endocrinology* 1994; **135:** 330–6.

203 MacIntyre I, Zaidi M, Alam A S, *et al.* Osteoclastic inhibition: an action of nitric oxide not mediated by cyclic GMP. *Proc Natl Acad Sci USA* 1991; **88:** 2936–40.

204 Ralston S H, Todd D, Helfrich M H, Grabowski P S, Benjamin N. Nitric oxide: a cytokine induced osteoblast product with stimulatory effects on bone resorption [abstract]. *Endothelium* 1993; **1**(suppl)**:** s10.

205 Beckerman K P, Rogers H W, Corbett J A, Schreiber R D, McDaniel M L, Unanue E R. Release of nitric oxide during the T cell-independent pathway of macrophage activation. Its role in resistance to Listeria monocytogenes. *J Immunol* 1993; **150:** 888–95.

206 Liew F Y, Millott S, Parkinson C, Palmer R M, Moncada S. Macrophage killing of Leishmania parasite in vivo is mediated by nitric oxide from L-arginine. *J Immunol* 1990; **144:** 4794–7.

207 James S L, Glaven J. Macrophage cytotoxicity against schistosomula of Schistosoma mansoni involves an L-arginine-dependent production of reactive nitrogen intermediates. *J Immunol* 1989; **143:** 4208–12.

208 Munoz-Fernandez M A, Fernandez M A, Fresno M. Synergism between tumor necrosis factor-alpha and interferon-gamma on macrophage activation for the killing of intracellular Trypanosoma cruzi through a nitric oxide-dependent mechanism. *Eur J Immunol* 1992; **22:** 301–7.

209 Rockett K A, Awburn M M, Cowden W B, Clark I A. Killing of Plasmodium falciparum in vitro by nitric oxide derivatives. *Infect Immun* 1991; **59:** 3280–3.

210 Flesch I E A, Kaufmann S H E. Mechanisms involved in mycobacterial growth inhibition. *Infect Immun* 1991; **59:** 2987–93.

211 Summersgill J T, Powell L A, Buster B L, Miller R D, Ramirez J A. Killing of Legionella pneumophila by nitric oxide in gamma-interferon-activated macrophages. *J Leukoc Biol* 1992; **52:** 625–9.

212 Karupiah G, Xie Q W, Buller R M, Nathan C, Duarte C, MacMicking J D. Inhibition of viral replication by interferon-gamma-induced nitric oxide synthase. *Science* 1993; **261:** 1445–8.

213 Malawista S E, Montgomery R R, van Blaricom G. Evidence for reactive nitrogen intermediates in killing of staphylococci by human neutrophil cytoplasts. A new microbicidal pathway for polymorphonuclear leukocytes. *J Clin Invest* 1992; **90:** 631–6.

214 Vincendeau P, Daulouede S. Macrophage cytostatic effect on Trypanosoma musculi involves an L-arginine-dependent mechanism. *J Immunol* 1991; **146:** 4338–43.

215 Sternberg J, McGuigan F. Nitric oxide mediates suppression of T cell responses in murine Trypanosoma brucei infection. *Eur J Immunol* 1992; **22:** 2741–4.

216 Clark I A, Rockett K A, Cowden W B. Possible central role of nitric oxide in conditions clinically similar to cerebral malaria. *Lancet* 1992; **340:** 894–6.

217 Calandra T, Baumgartner J D, Grau G E, *et al.* Prognosis values of tumour necrosis factor, interleukin-1, α-interferon and g-interferon in the serum of patients with septic shock. *J Infect Dis* 1990; **161:** 982–7.

218 Kilbourn R G, Jubran A, Gross S S, *et al.* Reversal of endotoxin-mediated shock by NG-methyl-L-arginine, an inhibitor of nitric oxide synthesis. *Biochem Biophy Res Commun* 1990; **172:** 1132–8.

219 Thiemermann C, Vane J. Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharides in the rat in vivo. *Eur J Pharmacol* 1990; **182:** 591–5.

220 Nava E, Palmer R M, Moncada S. Inhibition of nitric oxide synthesis in septic shock: how much is beneficial? *Lancet* 1991; **338:** 1555–7.

221 Vallance P, Moncada S. Effect of nitric oxide inhibitors on hypotension in patients with septic shock. *Lancet* 1991; **338:** 1557–8.

222 Schilling J, Cakmakei M, Battig U, Geroulanos S. A new approach in the treatment of hypotension in human septic shock by N^G-monomethyl-L-arginine, an inhibitor of the nitric oxide synthetase. *Intens Care Med* 1993; **19:** 227–31.

223 Petros A, Lamb G, Leone A, Moncada S, Bennett D, Vallance P. Effects of a nitric oxide synthase inhibitor in humans with septic shock. *Cardiovasc Res* 1994; **28:** 34–9.

224 Rosenberg S A, Lotz M T, Yang J C, *et al.* Experience with the use of high dose interleukin-2 in the treatment of 652 cancer patients. *Ann Surg* 1989; **210:** 474–84.

225 Kilbourn R G, Owen-Schaub L, Griffith O W, Logothetis C. Interleukin-2 mediated hypotension in dogs is reversed by N^G-methyl-L-arginine an inhibitor of nitric oxide formation. *Proc Am Assoc Cancer Res* 1992; **33:** 328.

226 Lepoivre M, Flaman J M, Henry Y. Early loss of the tyrosyl radical in ribonucleotide reductase of adenocarcinoma cells producing nitric oxide. *J Biol Chem* 1992; **267:** 22994–3000.

227 Mills C D, Shearer J, Evans R, Caldwell M D. Macrophage arginine metabolism and the inhibition or stimulation of cancer. *J Immunol* 1992; **149:** 2709–14.

228 Thomsen L L, Ching L M, Baguley B C. Evidence for the production of nitric oxide by activated macrophages treated with the anti-tumour agents flavone-8-acetic acid and xanthenone-4-acetic acid. *Cancer Res* 1990; **50:** 6966–70.

229 Andrade S P, Hart I R, Piper P J. Inhibitors of nitric oxide synthase selectively reduce flow in tumour-associated neovasculature. *Br J Pharmacol* 1992; **105:** 184–90.

230 Wink D A, Kasprzak K S, Maragos C M, *et al.* DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. *Science* 1991; **254:** 1001–3.

231 Nguyen T, Brunson D, Crespi C L, Penman B W, Wishnok J S, Tannenbaum S R. DNA damage and mutation in human cells exposed to nitric oxide in vitro. *Proc Natl Acad Sci USA* 1992; **89:** 3030–4.

232 Fehsel K, Jalowy A, Qi S, Burkart V, Hartmann B, Kolb H. Islet cell DNA is a target of inflammatory attack by nitric oxide. *Diabetes* 1993; **42:** 496–500.

233 Laval F, Wink D A. Inhibition by nitric oxide of the repair protein, *O*6-methylguanine-DNA-methyltransferase. *Carcinogenesis* 1994; **15:** 443–7.

234 Albina J E, Cui S, Mateo R B, Reichner J S. Nitric oxide-mediated apoptosis in murine peritoneal macrophages. *J Immunol* 1993; **150:** 5080–5.

235 Cui S, Reichner J S, Mateo R B, Albina J E. Activated murine macrophages induce apoptosis in tumor cells through nitric oxide-dependent or -independent mechanisms. *Cancer Res* 1994; **54:** 2462–7.

236 Messmer U K, Ankarcrona M, Nicotera P, Brune B. p53 expression in nitric oxide-induced apoptosis. *FEBS Lett* 1994; **355:** 23–6.

237 Persson M G, Gustafsson L E, Wiklund N P, Moncada S, Hedqvist P. Endogenous nitric oxide as a probable modulator of pulmonary circulation and hypoxic pressor response in vivo. *Acta Physiol Scand* 1990; **140:** 449–57.

238 Archer S L, Tolins J P, Raij L, Weir E K. Hypoxic pulmonary vasoconstriction is enhanced by the inhibition of an endothelium-derived relaxing factor. *Biochem Biophys Res Commun* 1989; **164:** 1198–205.

239 Crawley D F, Liu S F, Evans T W, Barnes P J. Endothelium-derived nitric oxide inhibits pulmonary vasoconstriction in isolated perfused rat lungs. *Am Rev Respir Dis* 1991; **143:** 32–7.

240 Adnot S, Raffestin B, Effahibi S, Braquet P, Chabrier P E. Loss of endothelium-dependent relaxant activity in the pulmonary circulation of rats exposed to chronic hypoxia. *J Clin invest* 1991; **87:** 155–62.

241 Dinh-Xuan A T, Higenbottam T W, Pepke-Zaba J, Clelland C A, Wallwork J. Reduced endothelium-dependent relaxation of cystic fibrosis pulmonary arteries. *Eur J Pharmacol* 1989; **163:** 401–3.

242 Dinh-Xuan A T, Higenbottam T W, Clelland C A, Pepke-Zaba J, Cremona G, Wallwork J. Impairment of pulmonary endothelium-dependent relaxation in patients with Eisenmenger's syndrome. *Br J Pharmacol* 1990; **99:** 9–10.

243 Dinh-Xuan A T, Higenbottam T W, Clelland C A, Pepke-Zaba J, Cremona G, Wallwork J. Impairment of endothelium-dependent pulmonary artery relaxation in chronic obstructive lung disease. *N Engl J Med* 1991; **324:** 1539–47.

244 Roberts J D, Polaner D M, Lang P, Zapol W M. Inhaled nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet* 1992; **340:** 818–9.

245 Kinsella J P, Neish S R, Shaffer E, Abman S H. Low-dose inhalation nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet* 1992; **340:** 819–20.

246 Rossaint R, Falke K J, Lopez F, Slama K, Pison U, Zapol W M. Inhaled nitric oxide for the adult respiratory distress syndrome. *N Engl J Med* 1993; **328:** 399–405.

247 Sanna A, Kurtansky A, Veriter C, Stanescu D. Bronchodilator effect of inhaled nitric oxide in healthy men. *Am J Respir Crit Care Med* 1994; **150:** 1702–4.

248 Leurs R, Brozius M M, Jansen W, Bast A, Timmerman H. Histamine H1-receptor-mediated cyclic GMP production in guinea-pig lung tissue is an L-arginine-dependent process. *Biochem Pharmacol* 1991; **42:** 271–7.

249 Jain B, Rubinstein I, Robbins R A, Leise K L, Sisson J H. Modulation of airway epithelial cell ciliary beat frequency by nitric oxide. *Biochem Biophys Res Commun* 1993; **191:** 83–8.

250 Warren J B, Loi R, Rendell N B, Taylor G W. Nitric oxide is inactivated by the bacterial pigment pyocyanin. *Biochem J* 1990; **266:** 921–3.

251 Allescher H D, Tougas G, Vergara P, Lu S, Daniel E E. Nitric oxide as a putative nonadrenergic noncholinergic inhibitory transmitter in the canine pylorus in vivo. *Am J Physiol* 1992; **262:** G695–702.

252 Vanderwinden J M, Mailleux P, Schiffman S N, Vanderhaeghan J, De Laet M. Nitric oxide synthase activity in infantile hypertrophic pyloric stenosis. *N Engl J Med* 1992; **327:** 511–5.

253 Vallance P, Moncada S. Hyperdynamic circulation in cirrhosis: a role for nitric oxide? *Lancet* 1991; **337:** 776–8.

254 Robert A, Leung F W, Kaiser D G, Guth P H. Potentiation of aspirin induced gastric lesions by exposure to cold in rats; role of acid secretion, mucosal blood flow and gastric mucosal prostanoid secretion. *Gastroenterology* 1989; **97:** 1147–58.

255 Pique J M, Whittle B J, Esplugues J V. The vasodilator role of endogenous nitric oxide in the rat gastric microcirculation. *Eur J Pharmacol* 1989; **174:** 293–6.
256 Walder C E, Thiemermann C, Vane J R. Endothelium-derived relaxing factor participates in the increased blood flow in response to pentagastrin in the rat stomach mucosa. *Proc R Soc Lond [Biol]* 1990; **241:** 195–200.
257 MacNaughton W K, Cirino G, Wallace J L. Endothelium-derived relaxing factor (nitric oxide) has protective actions in the stomach. *Life Sci* 1989; **45:** 1869–76.
258 Whittle B J, Lopez-Belmonte J, Moncada S. Regulation of gastric mucosal integrity by endogenous nitric oxide: interactions with prostanoids and sensory neuropeptides in the rat. *Br J Pharmacol* 1990; **99:** 607–11.
259 Miller M J, Zhang X J, Sadowska-Krowicka H, *et al.* Nitric oxide release in response to gut injury. *Scand J Gastroenterol* 1993; **28:** 149–54.
260 Boughton-Smith N K, Evans S M, Whittle B J. Elevated nitric oxide synthase activity in inflamed colon from a rat model of colitis. *Gut* 1992; **33** (suppl 2): S12.
261 Miller M J, Sadowska-Krowicka H, Chotinaruemol S, Kakkis J L, Clark D A. Amelioration of chronic ileitis by nitric oxide synthase inhibition. *J Pharmacol Exp Ther* 1993; **264:** 11–6.