Nitric oxide—from mediator to medicines

ABSTRACT—Nitric oxide is involved in a wide range of physiological processes in humans and in animals. It controls vascular tone, acts as a neurotransmitter and neuromodulator in the central and peripheral nervous systems and influences the activity of the immune system. Substances that selectively enhance or inhibit its synthesis or removal and modify its effects, are likely to yield interesting therapeutic agents.

The horseshoe crab *(Limulus polyphemus)*, a species that has been in existence for 500 million years, synthesises nitric oxide from L-arginine to prevent aggregation of its circulating haemocytes (Fig 1) [1]. The blood sucking insect *Rhodnius prolixus* injects nitric oxide bound to ferric iron into its prey to dilate blood vessels and inhibit platelet aggregation to make it easier to draw blood [2], and the starfish, an echinoderm, uses nitric oxide as a neuronal mediator of gut motility [3]. Mammals use this remarkable molecule for functions as diverse as controlling vascular tone, fighting infection and signalling within the central and peripheral nervous system [4]. The possibility exists that drugs based on the discovery of the L-arginine:nitric oxide pathway will be developed to alter blood pressure, cardiac function or gastrointestinal motility, modify the immune system and inflammatory response, or interfere with the processes of neuronal damage, seizure activity or memory formation. This article examines evidence that nitric oxide is involved in human physiology and pathophysiology and identifies likely therapeutic targets.

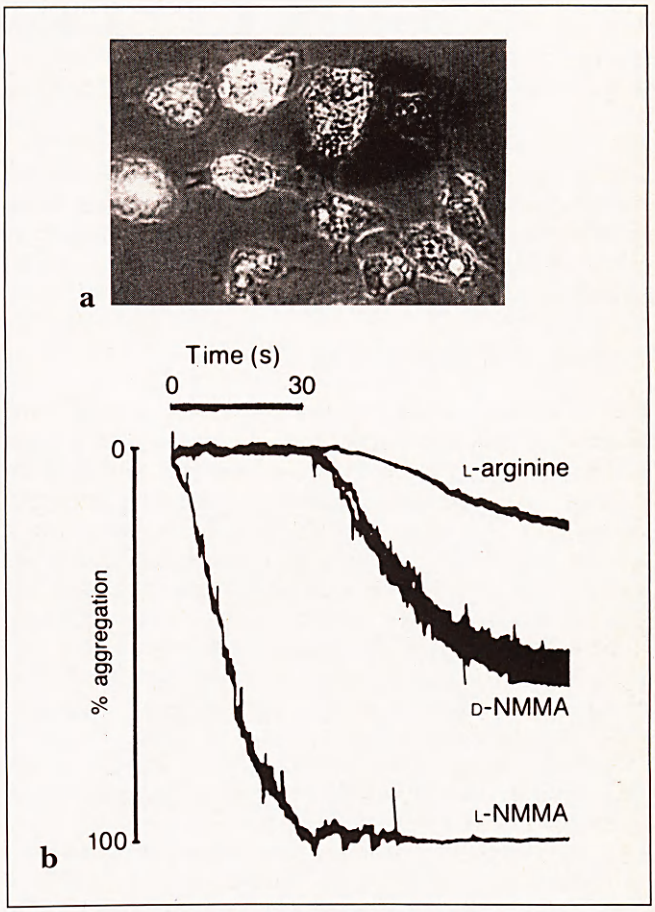

Fig 1. *The circulating haemocytes* (a) *of the horseshoe crab* (Limulus polyphemus) *have immunological and haemostatic properties. Aggregation of haemocytes* (b) is enhanced by treatment of the crabs with the nitric oxide synthase inhibitor L-NMMA and inhibited by the substrate for nitric oxide synthesis, L-arginine. D-NMMA does not affect nitric oxide synthase activity and does not alter aggregation of the haemocytes. (Reproduced with permission from *Philosophical Transactions of the Royal Society of London* [1].)

Nitric oxide: physiological mediator and toxic radical

Nitric oxide is synthesised from the semi-essential amino acid L-arginine by the action of a unique family of isoenzymes known as *nitric oxide synthases* (Fig 2). These are large and complicated haem-containing cytochrome P450-like enzymes that possess oxidative and reductive domains and require multiple co-factors for full activity [4,5].

In humans, three genes encoding nitric oxide synthases have been identified; the gene for the neuronal type enzyme is located on chromosome 12, for the endothelial enzyme on chromosome 7, and for the inducible enzyme on chromosome 17 (Fig 2).

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Constitutive nitric oxide synthase

A nitric oxide synthase is present as a normal constituent of healthy endothelial cells, platelets, myocardium, endocardium and certain central and peripheral neurons, and the nitric oxide produced

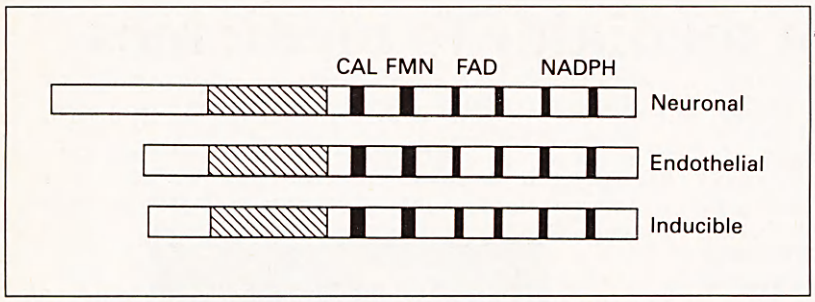

Fig 2. *Isoforms of nitric oxide synthase.* The gene for neuronal nitric oxide synthase is located on chromosome 12, for the endothelial enzyme on chromosome 7 and for the inducible enzyme on chromosome 17. Overall homology is around 50%. Shaded area represents putative arginine binding site.

acts as an intra- and inter-cellular messenger molecule. These enzyme types are activated by increases in intracellular calcium and make relatively small amounts of nitric oxide which produce changes in target cells, usually by activating soluble guanylate cyclase [4].

Inducible nitric oxide synthase

Inducible nitric oxide synthase is not a normal constituent of quiescent cells, but is expressed in a wide variety of cells (Table 1) after they are activated by exposure to products of infection including bacterial endotoxin [6] and exotoxin [7], or inflammatory cytokines including tumour necrosis factor, interferon gamma and interleukins 1 and 2 (for recent review see [8]). In many cells the activity of this enzyme appears to be independent of the intracellular level of free calcium or calcium/calmodulin. Once expressed, the enzyme continues to make large amounts of nitric oxide over periods of many hours or days. The nitric oxide activates guanylate cyclase and, in the quantities produced by the inducible enzyme, it has additional toxic effects: it interacts with iron–sulphur centred enzymes, impairs mitochondrial respiration [9,10], and also damages DNA [11]. Furthermore, reaction of nitric oxide with superoxide (O_2^-, another product of activated immune cells) may lead to the formation of more stable toxic radicals including peroxynitrite and hydroxyl anion [12]. Inducible nitric oxide synthase is part of the immune system.

Table 1. Tissues and cells in which formation of nitric oxide by the inducible nitric oxide synthase has been demonstrated.

Macrophage/monocytes
Vascular endothelial cells
Vascular smooth muscle cells
Myocardium/myocytes
Endocardium
Kupffer cells
Hepatocytes
Megakaryoblastic cells
Fibroblasts
Mesangial cells
Liver
Lung
Astrocytes

Enzyme inhibitors

L-Arginine is the endogenous substrate for nitric oxide synthase and the synthesis of nitric oxide can be competitively inhibited by guanidino-substituted arginine analogues including the naturally occurring compounds N^G-monomethyl-L-arginine (L-NMMA) and N^G,N^G-dimethylarginine (asymmetric dimethylarginine; ADMA) [4,13]. Inhibitors of nitric oxide synthase have been used to examine the role of nitric oxide in a wide variety of tissues *in vitro* and *in vivo,* in animals and in humans [4].

Distribution and effects of nitric oxide

Vasculature

Under normal physiological conditions the major source of nitric oxide within the cardiovascular system is the constitutive enzyme in the endothelium, and some vessels are also innervated by nitric oxide-releasing neurons (nitrergic nerves). Under certain pathological conditions, smooth muscle may be a source of nitric oxide (Fig 3).

Endothelium

Nitric oxide accounts for the biological activity of Furchgott's endothelium-derived relaxing factor (EDRF) [14], it dilates blood vessels and inhibits the adhesion of circulating cells to the endothelial lining. Nitric oxide synthase activity is present in the endothelium of human isolated arteries [15], arterioles [16], veins [17] and venules [18], and the enzyme has been isolated, sequenced and cloned from human umbilical vein endothelial cells [19,20].

Direct infusion of the nitric oxide synthase inhibitor L-NMMA into the brachial artery of healthy volunteers causes a substantial fall in resting forearm blood flow, indicating that continuous synthesis of nitric oxide is an important determinant of the basal tone of small arteries and arterioles [21]. Consistent with this obser-

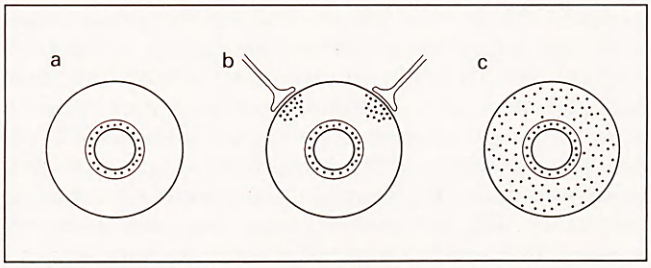

Fig 3. *Sources of nitric oxide in blood vessels.*
a) In most, if not all, vessels nitric oxide is synthesised within the endothelium.
b) In certain vessels (eg cerebral, adrenal, corpus cavernosum) nitric oxide is also synthesised by nerves in the adventitia (nitrergic nerves).
c) After exposure to endotoxin or cytokines, the inducible enzyme is expressed throughout the vessel wall and produces large amounts of nitric oxide.

vation, systemic injection of L-NMMA increases blood pressure in experimental animals [22,23] and healthy volunteers [24]. The precise physiological role of basal nitric oxide release is not yet known but its vasodilator action in the arterial circulation of the human forearm appears to be counteracted by the constrictor action of noradrenaline released from sympathetic nerves [21,25].

The level of activity of the constitutively expressed enzyme depends on the intracellular concentration of calcium/calmodulin and is increased by local hormones and autacoids including acetylcholine, bradykinin and substance P, which act on receptors located on the endothelial cell surface. Indeed, this is the mechanism by which these agents produce at least part of their vasodilator effects [4]. Acute exposure of endothelium to shear stress also elevates intracellular calcium and activates constitutive nitric oxide synthase. This accounts for the phenomenon of flow-mediated dilatation in certain vessels and influences the distribution of blood flow within tissues [26,27]. This type of flow- or shear-dependent autoregulation opposes 'classical' myogenic autoregulation. Increased nitric oxide production in response to acute changes in shear stress might contribute to the adaptive vasodilatation in response to rapid plasma expansion [28]. In contrast, chronic exposure to shear stress increases expression of the constitutive enzyme [29], an effect also produced by oestrogen [30]. Oestrogen- or chronic shear stress-induced increases in the expression of constitutive nitric oxide synthase could account for the physiological vasodilatation of pregnancy.

Nitric oxide synthesised by endothelium also inhibits platelet aggregation and attenuates adhesion of platelets and white cells [31,32]; the anti-aggregatory effects of nitric oxide are synergistic with those of another endothelium-derived mediator, prostacyclin [31]. These effects of nitric oxide are mediated, at least in part, by alteration in the expression of configuration of adhesion molecules on the platelet cell surface. Consistent with these studies *in vitro,* inhibition of nitric oxide synthesis *in vivo* promotes platelet accumulation in the lungs of rabbits treated with ADP [33] and increases platelet aggregation at sites of experimental arterial damage [34]. In contrast, short term administration of an inhibitor of nitric oxide synthase to healthy animals [35] or humans [36] does not cause generalised platelet activation.

After exposure to endotoxin or cytokines the endothelium also expresses the inducible isoform of nitric oxide synthase [37]. The greater generation of nitric oxide from this enzyme increases vascular relaxation, modifies the adhesion of platelets to the activated endothelium [37] and, under certain conditions, promotes cell damage [38]. The effects of inducible nitric oxide synthase in the cardiovascular system are discussed in further detail below.

Neurons

Experiments in animals suggest that certain neurons release nitric oxide onto the abluminal side of the blood vessel. Immunohistochemical studies in the rat demonstrate that the cerebral vasculature is densely innervated with these nitrergic neurons [39]. The same appears to be true for the human cerebrovasculature, although the functional significance of these neurons remains to be determined. In the peripheral vasculature, nitrergic innervation of the human corpus cavernosum has been demonstrated and may be important in the process of penile erection (see below).

Smooth muscle

Under normal physiological conditions, vascular smooth muscle does not synthesise nitric oxide. However, after exposure to endotoxin or cytokines, inducible nitric oxide synthase is expressed and the nitric oxide produced leads to profound vascular relaxation, resistance to conventional constrictor agents, and might cause vascular damage (for recent review see [8]; Fig 3). Messenger RNA for inducible nitric oxide synthase has recently been found in human cultured smooth muscle cells incubated with a cytokine/endotoxin mixture [40] and certain human blood vessels incubated with endotoxin or cytokines demonstrate depressed contractions to vasoconstrictors which are normalised by incubation with a nitric oxide synthase inhibitor [41,42]. Furthermore, patients with sepsis [43] have elevated circulating levels of nitrate, a stable breakdown product of nitric oxide, and inhibition of nitric oxide synthesis with even low doses of L-NMMA causes a substantial increase in blood pressure [44].

Heart

Constitutive nitric oxide synthase is present in myocardium and endocardium, and exogenously administered nitric oxide shortens contraction time and may be negatively inotropic [45,46]. Human atrium contains constitutive nitric oxide synthase [47] and in the cardiomyopathic ventricle the inducible isoform is expressed [47]. Preliminary evidence suggests that nitric oxide donor drugs (eg glyceryl trinitrate or sodium nitroprusside) may directly decrease myocardial contraction [48] and nitric oxide synthase inhibitors may improve contractility. However, when these agents are administered systemically *in vivo* their effects are more complex and include direct and indirect actions. Inhibition of endogenous nitric oxide synthesis usually leads to a fall rather than a rise in cardiac output [44,49]; this is probably due to reflex changes that occur when peripheral resistance and blood pressure increase.

Platelets

The L-arginine:nitric oxide pathway is present in human platelets [50]. The nitric oxide synthase is activated by the increases in intracellular calcium that occur during platelet activation and the nitric oxide synthesised may act as a negative feedback system to limit the extent of activation [30,50].

Nitrergic nerves

In addition to those found in the cardiovascular system, neurons staining for nitric oxide synthase have been found in the lung, both in the vasculature and in the bronchial tree, in the genitourinary tract and in the gut. Nitric oxide is released from many nerves previously classified as non-adrenergic, non-cholinergic or 'NANC' nerves.

In the gastrointestinal system, release of nitric oxide in response to nerve stimulation mediates adaptive relaxation of the stomach (the process by which the stomach accommodates food) [51], relaxation of sphincters (including the sphincter of Oddi) [52] and the relaxant part of the peristaltic cycle [53]. In human gut, nitrergic neurons have been demonstrated in the myenteric plexus [54], sphincter of Oddi [52] and duodenal sphincter [55].

In human bronchi *in vitro*, neuronally mediated bronchodilatation is inhibited by nitric oxide synthase inhibitors [56] and the finding that healthy humans exhale nitric oxide [57] suggests a possible role for this mediator in lung physiology, most probably in the process of matching ventilation and perfusion [58].

Genitourinary system

Nitric oxide relaxes smooth muscle of the upper and lower urinary tract [59], uterus [60] and corpus cavernosum [61]. In the urinary tract, nitrergic neurons may have a particular role in the control of bladder outflow [59]. Nitric oxide synthase activity has been detected in rat and human uterus [60], and infusion of a nitric oxide donor stops labour in sheep [62]. It has been proposed that basal production of nitric oxide maintains the uterus in a quiescent state during pregnancy [63] but further experiments are required to confirm these findings. Immunohistochemistry has identified neurons staining for nitric oxide synthase in human corpus cavernosum [64] and functional studies demonstrate that neurogenic relaxation of this tissue is inhibited by nitric oxide synthase inhibitors [61]. Nitric oxide donors have been reported to promote erection in patients [65] and L-NMMA blocks erection in experimental animals [66].

In the kidney nitric oxide may have a local signalling role; in the rat kidney the macula densa synthesises nitric oxide in response to sodium reabsorption and this dilates the afferent arteriole to increase glomerular filtration rate [67].

Endocrine system

Nitric oxide has been reported to increase the release of insulin from the pancreas [68], inhibit the release of renin from the kidney [69] and might be involved in the regulation of thyroid hormone production [70]. Furthermore, the adrenal gland is densely innervated with nitrergic nerves [39]. Cyclic GMP has been implicated in the control of hormone secretion and a role for nitric oxide in modulating levels of cyclic GMP in endocrine glands seems likely. The substrate for nitric oxide, L-arginine, has been used for many years for testing pituitary function and stimulates the release of a variety of hormones including growth hormone, prolactin, insulin, glucagon, somatostatin, pancreatic polypeptide and catecholamines [71]. However, the direct contribution made by nitric oxide to the responses seen is uncertain: other amino acids also stimulate hormone release and D-arginine (which is not a substrate for nitric oxide synthase) appears to be as effective as L-arginine at increasing insulin levels [72].

Central nervous system

Neurons containing nitric oxide synthase are distributed throughout the brain and are present in abundance in the cerebellum, superior and inferior colliculi and the granule cell layer of the olfactory bulb [39]. Nitrergic neurons are also located in the cerebral cortex, hippocampus, posterior pituitary and autonomic fibres in the retina. Stimulation of the excitatory *N*-methyl-D-aspartate (NMDA) glutamate receptor leads to the release of nitric oxide [73].

Nitric oxide is an important mediator of cell–cell signalling and may act as a retrograde messenger,

allowing the post-synaptic cell to send signals back to the pre-synaptic neuron. Nitric oxide synthase has been localised in human brain [74]. In the central nervous system three major physiological roles for nitric oxide have been proposed: as a mediator of long term depression [75] and potentiation [76], the fundamental mechanisms of memory formation by which individual neurons 'remember' the signals they have previously received: as a mediator of short term electrocortical activation [77], an alerting response important in control of the arousal state: and as a modulator of pain perception [78,79]. In addition, nitric oxide may inhibit sympathetic outflow [80].

Immune function and inflammation

At the time when vascular biologists were pursuing the identity of EDRF, immunologists were demonstrating that macrophages synthesise nitrite and nitrate and exhibit L-arginine-dependent cytotoxicity [9]. Nitric oxide provided the answer to both puzzles [81,82]. There is now overwhelming evidence that nitric oxide synthesised by inducible nitric oxide synthase in activated murine macrophages is an important host-defence mechanism. It is involved in the killing of pathogens including leishmania, mycobacterium tuberculosis, malaria parasites and certain fungi, mediates 'non-specific' immunity, and is toxic to tumour cells (for recent reviews see [4,82]). Inhibition of nitric oxide synthesis facilitates the replication of leishmania in the mouse *in vivo* [83].

In addition to its direct toxic effects on pathogens, tumour cells and host cells, nitric oxide regulates lymphocyte function. Nitric oxide released from macrophages leads to suppression of lymphocyte function and may have a particular role in inhibiting certain subsets of T helper cells [84]. Other cells of the immune system also synthesise nitric oxide. There is evidence that lymphocytes [85] and neutrophils synthesise [86] and release nitric oxide, although the precise role of the mediator for the normal functioning of these cells remains to be determined.

Despite the abundance of data from studies using animal tissues, the importance of the L-arginine:nitric oxide pathway for the function of human macrophages is still uncertain. Nitrite and nitrate (stable breakdown products of nitric oxide) are produced by human macrophages, but the amounts are variable and less than those produced by murine cells. Injection of interleukin-2 into patients markedly raises plasma nitrate derived from L-arginine [87], and patients with sepsis have high circulating levels of nitrogen oxides [43]. However, the precise source of nitric oxide has yet to be established and there is no direct evidence that human immune cells produce cytotoxic amounts of nitric oxide or that nitric oxide synthase inhibitors have an immunosuppressive effect in humans.

Nitric oxide and disease states

A mediator as ubiquitous as nitric oxide is likely to be involved in a wide variety of disease processes. In the cardiovascular system, decreased synthesis or action of nitric oxide has been implicated in virtually every disease associated with increased vascular tone, vasospasm or enhanced adhesion of platelets and white cells to the vessel wall. Diminished endothelium-dependent dilatation or decreased basal nitric oxide-mediated vasodilatation has been demonstrated in patients with hypertension (Fig 4) [88,89], diabetes [90], hypercholesterolaemia [72] and atheroma [91]. In the cerebral circulation, disorders of endothelium- or neuronally derived nitric oxide have been implicated in the pathogenesis of migraine [92] and vasospasm after subarachnoid haemorrhage [93]. In the heart, increased production of nitric oxide following expression of the inducible isoform of nitric oxide synthase could contribute to the pathogenesis of cardiomyopathy and ventricular dysfunction [47].

In the gut, loss of nitrergic nerves occurs in infantile hypertrophic pyloric stenosis [55] and in patients with achalasia [54], and diminished nitric oxide production might underlie opiate-induced constipation [94]. In the respiratory system, loss of endothelium-derived nitric oxide in the pulmonary vessels provides a link between chronic lung disease and pulmonary hypertension [95]. Abnormalities of nitrergic neurons supplying the airways could increase bronchial constriction; and in the genitourinary system, deficient nitric oxide-mediated relaxation of the corpus cavernosum may be associated with impotence [96]. In patients with renal failure, accumulation of an endogenous inhibitor of nitric oxide synthesis, ADMA, could contribute to hypertension or immune dysfunction [13] while over-production of nitric oxide synthesis in platelets has been suggested as a cause of the bleeding tendency of uraemia [35,97].

In the central nervous system, inhibition of nitric oxide synthesis impairs learning in rats, induces somnolence in sheep [98], enhances the action of certain anaesthetic agents [98,99], protects against epilepsy, and reduces or increases damage caused by stroke depending on the experimental model [100–103]. The interpretation of these results is often complicated by the increase in blood pressure that accompanies systemic inhibition of nitric oxide synthesis but the data suggest possible roles for nitric oxide in disease processes in the brain. Evidence in humans is hard to come by, but neurons staining for NADPH diaphorase (a stain that detects nitric oxide synthase) are spared in Huntington's chorea [104] and it has been proposed that over-production of nitric oxide could contribute to programmed or pathological cell death in the central nervous system. Indeed, nitric oxide has been implicated in cerebral damage produced by stroke, Parkinson's disease and AIDS dementia [103,105].

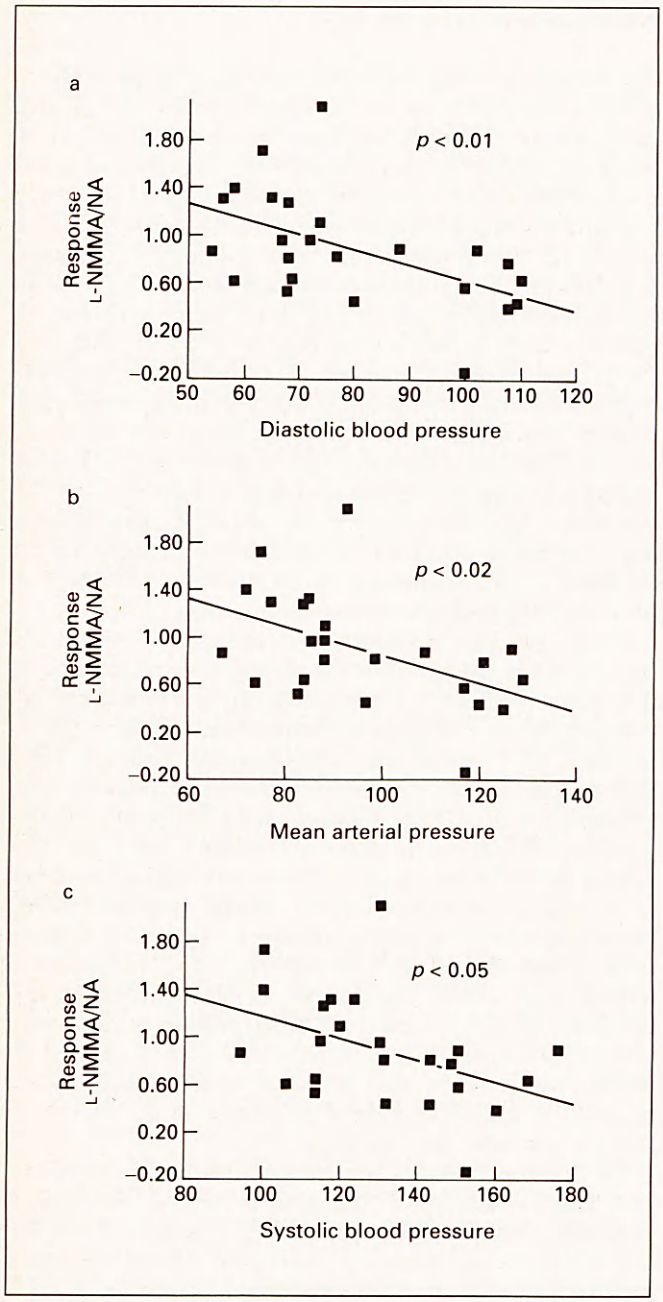

Fig 4. *The constrictor response to L-NMMA is due to inhibition of nitric oxide synthase by this compound.* The response to L-NMMA decreases as blood pressure increases, indicating that the L-arginine:nitric oxide pathway contributes less to basal vasodilator tone in hypertension. (Reproduced with permission from the *Journal of Hypertension* [88].)

Induction of nitric oxide synthase in response to cytokines or endotoxin appears to be part of the inflammatory response and could contribute to vasodilatation, vascular leakage and tissue damage in a number of inflammatory conditions. There is evidence for induction of nitric oxide synthase in the joints of patients with rheumatoid arthritis [106], the gut of patients with ulcerative colitis [107,108], in the ventricles of patients with cardiomyopathy [47] and in animals with hypercholesterolaemia [109] or immune complex glomerulonephritis [110]. In patients with septic shock, nitric oxide synthesis is enhanced [43], and injection of L-NMMA reverses the hypotension [44,111]. Nitric oxide has joined the list of mediators involved in the process of local and systemic inflammation in humans. Finally, an inducible nitric oxide synthase, expressed after treatment with interleukin-1ß, has been cloned from human chondrocytes [112].

Therapeutic possibilities

The ubiquity of nitric oxide and its involvement in such a wide variety of physiological and pathophysiological processes suggests that drugs designed to alter its biological activity might have diverse effects. A major problem with such treatments is lack of selectivity for particular cells or tissues. The targeting of drugs that alter the production of nitric oxide to specific tissues poses a challenge for drug development. Parallels may be drawn with other widespread mediators such as serotonin which has spawned a generation of drugs with potential indications for the treatment of hypertension, thrombosis, depression, nausea and vomiting, dementia, migraine or addictive behaviour [113]. As drugs based on the L-arginine:nitric oxide pathway are developed they will help to clarify the role of nitric oxide in the seemingly endless list of diseases in which it has been implicated as an important mediator [114].

Drugs to increase the production or effect of nitric oxide

Nitric oxide donors

One class of drugs that acts as a nitric oxide donor has been in clinical use for over a century; nitric oxide is the active moiety of nitrovasodilators including glyceryl trinitrate, sodium nitroprusside and newer compounds such as molsidomine. The vascular effects of these compounds are well established but knowledge of the endogenous production of nitric oxide has given new insight into their mechanism of action and suggested possible novel uses for these drugs.

Nitrovasodilators preferentially dilate veins and this is the basis for at least part of the efficacy of glyceryl trinitrate in the management of heart failure and angina and explains the unwanted effect of postural hypotension. The venoselectivity is most easily explained by the observation that veins have a low basal output of nitric oxide [17] and consequently the guanylate cyclase in venous smooth muscle is up-regulated. Similarly, evidence from studies in animals suggests that nitrovasodilators have an exaggerated effect

in vessels with damaged endothelium or impaired nitric oxide synthesis [115]. If this effect also occurs in human coronary vessels *in vivo* it might contribute to the anto-anginal effect of nitrates.

Anti-platelet actions of nitrovasodilators have been demonstrated in some studies, but the effect is relatively small compared with the dilatation. However, targeting the delivery of nitric oxide to platelets produces a different profile. *S*-nitrosoglutathione is metabolised to nitric oxide within platelets and, unlike the existing nitrovasodilators, this compound markedly inhibits platelet aggregation at doses that cause only minimal vasodilatation [116,117]. Certain experimental models suggest a possible protective role for nitrovasodilators in stroke, but it is not yet clear whether this is a vascular, platelet or neuronal effect of the drugs.

Non-cardiovascular indications for nitric oxide donors are also being explored. Inhaled amyl nitrite was first used to treat asthma in 1866 [118] and there is now renewed interest in the use of nitric oxide donors to treat a variety of respiratory diseases. Inhalation of very low concentrations of nitric oxide gas (in the order of 100 parts per billion) appears to cause selective dilatation of vessels supplying ventilated alveoli and this approach might be of use in certain patients with acute lung injury [119] or other causes of pulmonary hypertension [120,121]. Targeting nitric oxide to respiratory, gastrointestinal or genitourinary smooth muscle might lead to a new generation of nitric oxide donors with wide-ranging therapeutic indications.

Agonist-stimulated nitric oxide synthesis

Hormones and autacoids, including acetylcholine, bradykinin, substance P, adrenaline and serotonin, stimulate endothelial cells to release nitric oxide; in the brain, glutamate and NMDA have similar effects. Whether agonists could be developed to cause long term stimulation of the L-arginine:nitric oxide pathway remains to be determined. The increase in bradykinin levels that occurs when kininase II (better known as angiotensin-converting enzyme) is inhibited by drugs such as captopril or enalapril, stimulates nitric oxide release from endothelium and this has been implicated as an additional mechanism of action of these agents [122].

Protection of nitric oxide

Superoxide rapidly inactivates nitric oxide [123] and it might be possible to alter nitric oxide homeostasis by giving superoxide dismutase or blocking the endogenous production of superoxide.

Induction of nitric oxide synthase

Certain types of immunotherapy may induce nitric oxide synthase; injection of interleukin-2 for the treatment of renal tumours increases the production of nitrite and nitrate [87]. It is not yet known whether the enhanced nitric oxide synthesis contributes to the anti-tumour effects of this agent but this is a possibility. One unwanted effect of interleukin-2 is hypotension and this does seem to be due to the enhanced nitric oxide synthesis.

Provision of substrate (arginine)

Infusion of L-arginine lowers blood pressure in healthy volunteers and patients with hypertension [124], and corrects endothelial dysfunction in patients with hypercholesterolaemia [72,125]. However, large amounts of the amino acid have to be given intravenously and certain of its effects appear to be independent of nitric oxide production [126]. The potential effects of dietary arginine supplementation in cardiovascular disease are currently under investigation.

Drugs to decrease nitric oxide production or effect

In certain situations it may be desirable to inhibit the synthesis or action of nitric oxide to reverse hypotension, prevent the toxic effects of large quantities of the radical or inhibit cortical arousal or epileptiform activity.

Substrate analogues

Inhibition of nitric oxide synthases with substrate analogues such as L-NMMA reverses the local vasodilatation associated with inflammation or the profound hypotension seen in experimental models of septic shock [4,8] and protects against the cytotoxic effects of endotoxin and certain cytokines *in vitro* [37]. In patients with septic shock, L-NMMA restores blood pressure [44,111] although its effects on tissue damage, morbidity and mortality are unknown. Selective inhibitors of the inducible isoform of nitric oxide synthase are currently being developed. They have the theoretical advantage that they would inhibit the pathophysiological production of nitric oxide without affecting normal endothelial, neuronal or platelet function.

Selective inhibitors of cerebral nitric oxide synthase have been described [127]. These should provide useful tools to study the physiological role of the pathway in the central nervous system and might provide novel therapeutic approaches to the treatment of epilepsy or neuronal damage following stroke.

Inhibition of induction or co-factor synthesis

Anti-inflammatory glucocorticoids inhibit induction of nitric oxide synthase but are ineffective once the enzyme is expressed [128]. The anti-inflammatory and cytotoxic drug methotrexate has a similar profile but

acts by inhibiting the synthesis of tetrahydrobiopterin, an essential co-factor for nitric oxide synthase induction and activity [129]. It is not yet known how these effects relate to the therapeutic efficacy of these drugs, but the findings raise the possibility of developing more specific anti-inflammatory or immunosuppressive agents based on modification of the inducible nitric oxide pathway.

Conclusions

The formation of nitric oxide from L-arginine is a ubiquitous biochemical pathway. Experiments in animals have demonstrated that it is important in the control of vascular tone and platelet and white cell function, is the mediator released by nerves previously classified as 'non-adrenergic, non-cholinergic nerves' that influence gut motility and airways calibre, and is a neurotransmitter and neuromodulator in the central nervous system. In addition to its signalling role, nitric oxide is a toxic mediator utilised for host defence, contributing to the local and systemic inflammatory response, and potentially damaging to host cells. Some of these functions have also been clearly defined using human tissue or in humans *in vivo* [130]; for others, including host defence and the action of nitric oxide in the central nervous system, their roles in human physiology or pathophysiology have yet to be clearly identified. As drugs are developed that selectively enhance or inhibit the pathways, the contribution made by the L-arginine:nitric oxide pathway to biological processes in health and disease will become clearer and new therapies will doubtless emerge.

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