The biological significance of nitric oxide formation from L-arginine

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Introduction

The vascular endothelium synthesizes nitric oxide (NO) from the terminal guanidino nitrogen atom(s) of L-arginine. NO, derived from endothelium-dependent relaxing factor (EDRF), is becoming apparent that the synthesis of NO from L-arginine not only explains endothelium-dependent vascular relaxation, but is also widespread mechanism for the regulation of cell function and communication. The evidence for this so far derives from work on vascular endothelial cells, macrophages, the central nervous system and neutrophils, although it is likely that this mechanism is also present in other tissues. This review will outline the evidence for this hypothesis and will consider its physiological and pathological implications.

The vascular endothelium

EDRF is a labile humoral substance released from the vascular endothelium which relaxes vascular smooth muscle and inhibits platelet aggregation and adhesion via stimulation of the soluble guanylate cyclase (for reviews see [1, 2]). It has now been identified as NO [3-5]. Furthermore, vascular endothelial cells in culture synthesize NO from the terminal guanidino nitrogen atom(s) of L-arginine [6, 7]. This reaction, which forms L-citrulline as a co-product and is NADPH-dependent, is specific, since other analogues of L-arginine, including its D-enantiomer, are not substrates. In addition, one analogue, N\textsuperscript{\textomega}-monomethyl L-arginine (L-NMMA), inhibits the synthesis in a dose-dependent and enantiomerically specific manner [9].

In rabbit aortic rings [10] and guinea-pig pulmonary artery rings [11], L-NMMA induces a small but significant endothelium-dependent contraction and inhibits the relaxation and the release of NO induced by acetylcholine (ACH). In the coronary circulation of the rabbit isolated heart, L-NMMA increases coronary perfusion pressure and inhibits ACH-induced release of NO and fall in coronary perfusion pressure [12]. More significantly, in the anaesthetized rabbit, L-NMMA induces a dose-dependent, long-lasting increase in mean arterial blood pressure and inhibits the hypotensive action of ACH without affecting that of the endothelium-independent vasodilator, glyceroltrinitrate [13]. All these actions of L-NMMA can be reversed by L-arginine in a way which suggests a competition between the inhibitor and the substrate [8].

These results indicate that there is, in the vasculature, a continuous utilization of L-arginine for the generation of NO and that this NO plays a role in the control of vascular tone and blood pressure. The release of NO by the vascular endothelium may play other important regulatory roles in the cardiovascular system, including inhibition of platelet aggregation [14] and adhesion [15], modulation of mesangial cell contractility [16] and control of the release of renin [17]. Whether NO is also involved in the modulation of smooth muscle cell replication or in the control of enzymes regulating cholesterol metabolism requires investigation.

Abbreviations used: EDRF, endothelium-derived relaxing factor; L-NMMA, N\textsuperscript{\textomega}-monomethyl L-arginine; ACH, acetylcholine; LPS, lipopolysaccharide; NMDA, N-methyl-D-aspartate; NO, nitric oxide.

The macrophage

Mammals have been shown to be capable of synthesizing NO\textsuperscript{\textomega} [18]. Treatment of animals with Escherichia coli lipopolysaccharide (LPS) increases blood and urinary excretion of NO\textsuperscript{\textomega} [19, 20] and exposure of macrophages to LPS in vivo increases their production of NO\textsuperscript{\textomega} and NO\textsuperscript{\textomega} [20], with the formation of L-citrulline as a co-product [21]. The NO\textsuperscript{\textomega} and NO\textsuperscript{\textomega} formed are derived from the terminal guanidino nitrogen atom(s) of L-arginine [21] and NO has recently been identified as an intermediate in this pathway [22]. This L-arginine-dependent pathway, which is distinct from arginase, has been shown to contribute to the microbial and tumoricidal activities of macrophages, which include inhibition of mitochondrial respiration, aconitase activity and DNA synthesis. These activities as well as the generation of NO\textsuperscript{\textomega} and NO\textsuperscript{\textomega}, are inhibited by L-NMMA [22-24].

The functions of the generation of NO by macrophages, other than as a cytotoxic mechanism, are not known. It is possible, however, that NO may regulate cyclic GMP levels in cells with which the macrophage comes in contact. Furthermore, generation of NO may alter the intracellular environment of the macrophage by the induction of specific changes in metabolism which facilitate its microbistatic activity [24]. Whether this is subsequent to the elevation of cyclic GMP or is an independent mechanism requires investigation.

Since NO is released by inflammatory cells and vascular tissue and exerts powerful biological actions it is likely that it also plays a role as a mediator of inflammation.

The central nervous system

In 1977, the soluble fraction of brain synaptosomes was shown to contain a low-molecular-mass activating factor of the soluble guanylate cyclase [25]. This factor, whose action was prevented by haemoglobin, was subsequently identified as L-arginine [26]. We have recently shown that in a rat brain synaptosomal preparation L-arginine is converted to L-citrulline with a concomitant increase in cyclic GMP [27]. Both of these processes are inhibited by L-NMMA, indicating that the enzyme system involved is similar to that in the endothelial cell and in the macrophage [8, 9, 23].

Recently, N-methyl-D-aspartate (NMDA), an excitatory amino acid known to elevate cyclic GMP levels in the brain, has been shown to induce the release of an EDRF-like material from rat cerebellar cells. The release of this factor, which is Ca\textsuperscript{\textomega}+-dependent, accounts for the elevation in cyclic GMP levels that follows NMDA receptor activation [28].

The soluble guanylate cyclase is not uniformly distributed throughout the brain and its role is yet to be understood. Cholinergic and adrenergic stimulation, excitatory amino acids, some other amides, depolarizing agents, peptides, nitroso compounds and NO have all been shown to elevate cyclic GMP in the central nervous system (for review see [29]). It is possible that in many of these cases receptor-mediated activation of the formation of NO from L-arginine leads to stimulation of the soluble guanylate cyclase, resulting in a variety of biological consequences.

The l-arginine: NO-forming enzyme system

Although the precise details of the mechanisms involved remain to be elucidated, the existing evidence warrants the conclusion that L-arginine is converted into NO with the concomitant formation of L-citrulline.

The tissues from which most information is at present derived are the vascular endothelium, the macrophage and...
the central nervous system. In all three, the enzyme(s) involved is soluble, NADPH-dependent, requires a divalent cation, and is inhibited by L-NMMA [8, 23, 27].

Circumstantial evidence already points to the formation of NO from L-arginine in other tissues (see Table 1). NO formation has recently been demonstrated in neutrophils [30] and we have found that this formation is inhibited by L-NMMA [31]. The effect of L-NMMA is reversed by L-arginine [31]. Furthermore, EMT-6 adenocarcinoma cells, treated with supernatant of activated macrophages, generate L-citrulline, NO₂, and NO₃ from L-arginine [32]. Whether or not the recently described formation of L-citrulline from L-arginine in murine hematopoietic cells occurs via this pathway has not yet been established [33].

When analysed in terms of substrate specificity, products and cofactor requirements, the similarities between the systems from different sources is remarkable (see Table 1). At present it is not yet possible to tell whether they are the same enzyme or a system of isoenzymes. The latter possibility is more likely since the enzymes from the different cells show small variations in their substrate specificity and in their susceptibility to inhibition by L-arginine analogues.

The exact mechanism whereby the terminal guanido nitrogen atom(s) of L-arginine is liberated and subsequently oxidized to NO is not known. In the macrophage, an L-arginine deiminase that liberates ammonia, which is then oxidized to NO₂ and NO₃ has been postulated [34]. So far the term deiminase has been used for a reaction in which the imino nitrogen is removed, without a rigorous identification of the enzyme. However, deiminases have only been clearly identified in bacteria [35] and other mechanisms such as N oxidation, which has recently been proposed [22], may be involved. Furthermore, the NADPH-dependence of the enzymes suggests a redox mechanism. Additional work will be required to clarify this process.

The release of EDRF is known to be dependent on extracellular calcium [36]. The way in which this relates to the requirement for divalent cations by the enzyme(s) involved in the generation of NO from L-arginine also requires elucidation.

Conclusions

Present evidence allows us to postulate that the formation of NO from L-arginine is a widespread transduction mechanism for activation of the soluble guanylate cyclase, leading to a variety of functions in different cells.

Table 1. The L-arginine: NO-forming system in different cells and tissues

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Inhibitor</th>
<th>EMT-6</th>
<th>PMN</th>
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<tbody>
<tr>
<td>L-arginine</td>
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</tr>
<tr>
<td>L-NMMA</td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td>Products</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>L-citrulline</td>
<td>ND</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NO</td>
<td>+</td>
<td>ND</td>
<td>+</td>
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<tr>
<td>NO₂ / NO₃</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
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<tr>
<td>Cofactor</td>
<td></td>
<td></td>
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<tr>
<td>NADPH</td>
<td>ND</td>
<td>+</td>
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Abbreviations: EC, endothelial cell; ECH, endothelial cell homogenate; Mo, macrophage; CNS, brain synaptosomes; EMT-6, murine adenocarcinoma cells; PMN, polymorphonuclear leukocytes; +, active; ND, not done.

It has been proposed that NO activates the soluble guanylate cyclase by interaction with the iron atom in the haem of the enzyme. Furthermore, NO also binds to and oxidizes the Fe²⁺ in haemoglobin. Whether all the biological actions of NO are mediated through an interaction with transition metals, or whether its nitrosating ability also contributes to its actions, has yet to be established.

Administration of l-arginine induces the release of a number of hormones from the pancreas, the pituitary and the adrenal glands. It increases skin allograft rejection, increases tumour regression and decreases tumour recurrence and it also decreases cholesterol levels and atherogenesis in animals and man (for review see [37]). It is likely that at least some of these actions of L-arginine are mediated via the formation of NO. The use of L-NMMA, a specific inhibitor of the L-arginine: NO pathway in vitro and in vivo, will help to identify which of the actions of L-arginine are mediated via this pathway.

The precise distribution of this pathway in the body remains to be studied and for this a systematic investigation of the biological actions of L-arginine and the correlation between this and the distribution of the soluble guanylate cyclase in tissues will be necessary.

References

The first step in the process of virus infection is the binding of the antigen 4; sCD4, soluble CD4; VSV. vesicular stomatitis virus. followed by fusion of the outer coat of the virus and penetration of HIV. the mapping of functional epitopes of vesicular stomatitis virus (VSV) where the VSV particle is enveloped in HIV glycoproteins (Dalgleish et al., 1984). Such pseudotype particles will only plate on cells bearing CD4 antigen, but once uncoated will replicate as VSV, affording a simple plaque assay for receptors and virus penetration.

While mouse cells expressing human CD4 antigen allow HIV binding, they are not permissive to subsequent steps in virion penetration (Maddon et al., 1986). CD4+ mouse cells neither form syncitia on exposure to HIV nor plate VSV(HIV) pseudotypes. It appears that binding to the CD4 antigen is not sufficient to trigger virus internalization or cell-cell fusion. We still do not know what that trigger is. With many enveloped viruses, low pH, as found in endosomes, causes a conformational change in the viral glycoproteins exposing a fusion domain (Marsh, 1984). HIV entry and membrane fusion is not pH-dependent (Stein et al., 1987; McClure et al., 1988) which helps to explain cell-cell fusion at neutral pH during formation of syncytia. Neither does HIV entry appear to depend on endocytosis of CD4, as mutation or truncation of the cytoplasmic (C-terminal) domain of the antigen prevents phorbol ester-mediated endocytosis does not affect sensitivity of cells to HIV infection (Bedinger et al., 1988; Maddon et al., 1988). It would therefore appear that some secondary event at the surface of human cells exposes a fusigenic domain of the viral glycoproteins after they have bound to CD4 (Haseltine, 1988; Sattentau & Weiss, 1988).

Mapping functional epitopes on viral and cellular antigens

The HIV env gene encodes a glycoprotein precursor (gp160) that is cleaved to form the outer virion envelope (gp120) and the transmembrane (gp41) glycoproteins. The production of recombinant proteins has revealed that gp120 binds to CD4 antigen with high affinity (Kowalski et al., 1987; Lasky et al., 1987). Mutational analysis indicates that the 40 amino acids at the C-terminal domain of the antigen preventing phorbol ester-mediated endocytosis does not affect sensitivity of cells to HIV infection (Bedinger et al., 1988; Maddon et al., 1988). It would therefore appear that some secondary event at the surface of human cells exposes a fusigenic domain of the viral glycoproteins after they have bound to CD4 (Haseltine, 1988; Sattentau & Weiss, 1988).