EDITORIAL

Does Endothelium-Derived Nitric Oxide Have a Role in Cytokine-Induced Hypotension?

Carl F. Nathan, * Dennis J. Stuehr

Early studies of mammalian nitrogen oxide biosynthesis began with the rationale that endogenously formed nitrogen oxides could generate N-nitrosamines and, thus, initiate carcinogenesis (1). Since then, our understanding of the biologic consequences of nitrogen oxide biosynthesis has expanded considerably. Following its initial discovery in macrophages (2), nitrogen oxide biosynthesis has been demonstrated in endothelial cells (3,4), neutrophils (5,6), brain cells (7-9), Kupffer’s cells (10), hepatocytes (11), an adenocarcinoma cell line (12), and the adrenal gland (13). Investigators using 15N in studies of macrophages and endothelial cells (14,15) confirmed that nitrogen oxides are formed from one of the two equivalent guanidino nitrogens of L-arginine. The primary products of this enzymatic oxidation are nitric oxide and L-citrulline (3,4,11,16-18). Nitric oxide is bioactive but decomposes rapidly in aerated solutions to form mixtures of nitrite and nitrate.

The biologic effects of endogenously generated nitric oxide depend in part on the flux of nitric oxide reaching the target cell. When nitric oxide is produced in small amounts, many of its effects are mediated through activation of soluble guanylate cyclase (19,20). This signal transduction pathway plays a central role in the dynamic control of vascular resistance, platelet function, and excitatory synaptic transmission (3,4,8,9,21). When produced in larger amounts, nitric oxide inhibits synthesis of DNA and protein by unknown mechanisms and inhibits oxidative phosphorylation by reacting with iron-sulfur metalloenzymes of the mitochondrial electron-transport chain (17,22-24). Macrophage-derived nitric oxide can mediate tumor cell cytostasis (17,24) and is involved in macrophage-mediated killing or inhibition of a variety of microbial pathogens (25-27).

Evidently, the enzymes responsible for nitric oxide synthesis may constitute a family containing at least two distinct types (table 1). Both type I and type II nitric-oxide synthase are soluble and utilize L-arginine and reduced NADPH (NADPH) as substrates. The type I enzyme is induced in cells within 4-18 hours after exposure to a discrete set of immunostimulators (microbial products and cytokines) (11,12,28-30), while expression of the type II enzyme appears to be constitutive; that is, it requires no induction. Once induced, the type I enzyme synthesizes nitric oxide at a constant rate (29) for relatively long periods (5-36 hr) and, in cell-free systems, requires no added divalent metal ions. In contrast, the type II enzyme synthesizes nitric oxide within seconds in response to ligand-receptor-coupling events at the cell surface, displays a strict dependence on calcium ions and calmodulin, and is sensitive to calmodulin inhibitors (7-9,13). The type I enzyme requires tetrahydrobiopterin as a cofactor (31,32), but the type II enzyme apparently does not (9,13). N6-monomethyl-L-arginine is far more potent than N6-nitro-L-arginine in inhibiting the type I enzyme, whereas with the type II enzyme, the opposite is true (Gross SS, Stuehr DJ, Griffith O: unpublished data). Recent evidence suggests that the type I enzyme contains an NADPH-dependent flavin-adenine dinucleotide flavoprotein1; it is not known whether this is also true for the type II enzyme. So far, only cells of mouse or rat origin have been shown to express the type I enzyme.

In this issue of the Journal, Kilbourn and Belloni (33) report the provocative observation that mouse brain endothelial cells can release nitrogen oxides in response to cytokines. Their findings are novel in two ways.

First, the prolonged release of nitrogen oxides from endothelial cells in response to cytokines seems to lead to the accumulation of larger amounts of nitrogen oxides than endothelial cells were previously believed to make. Second, the results suggest that endothelial cells can activate their nitric-oxide synthase, not only by means of the rapid response to agonists that is characteristic of endothelial cells, neutrophils, and cerebellar cells, but also via

Endothelial cells are now known to express cytokine receptors and to respond to cytokines in culture (34-36). The prolonged release of nitrogen oxides from these cells may be mediated by cytokines. The cytokines that induce the synthesis of nitric oxide in these cells are not yet known. The observation that endothelial cells can release nitrogen oxides in response to cytokines suggests that these cells may play a role in the development of hypotension in sepsis, endotoxemia, and other conditions in which cytokines are released.

Table 1. Nitric-oxide synthases: evidence for an enzyme family

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells</td>
<td>Macrophages</td>
<td>Cerebellar cells</td>
</tr>
<tr>
<td></td>
<td>Hepatocytes</td>
<td>Endothelium</td>
</tr>
<tr>
<td></td>
<td>Tumor cells</td>
<td>Neutrophils</td>
</tr>
<tr>
<td>Mode of activation</td>
<td>Inducible</td>
<td>Constitutive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agonist triggered</td>
</tr>
<tr>
<td>Inhibited by</td>
<td>Trifluoperazine</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>N6-L-arginine analogues*</td>
<td>NMA &gt; NNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NNA &gt; NMA</td>
</tr>
<tr>
<td>Dependent on</td>
<td>Calmodulin</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Tetrahydrobiopterin</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Flavine-adenine dinucleotide</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

*NMA = N6-methyl-L-arginine; NNA = N6-nitro-L-arginine.

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Received February 22, 1990; accepted February 23, 1990.

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Stuehr DJ, Kwon NS, Cho JC, et al.: manuscript submitted for publication.
the slow process used by macrophages, hepatocytes, and tumor cells. This hypothesis raises important questions: Is the same enzyme system regulated in two ways? Alternatively, are these the first cells found to contain both type I and type II nitric-oxide synthases? Still other explanations could be considered. For example, cytokines might induce cells in the mouse brain cell cultures to slowly release an autacoid that triggers endothelial cell type II nitric-oxide synthase in the conventional manner.

As Kilbourn and Belloni point out, their findings have a therapeutically important implication. If endothelial-derived nitric oxide accounts for the vascular leak syndrome that accompanies experimental administration of certain cytokines, then blocking the production of nitric oxide may permit such cytokines to be given in higher doses with less toxicity.

However, several points need clarification or qualification. The authors do not indicate whether their endothelial preparations could, in fact, release nitrogen oxides rapidly in response to agonists like histamine, acetylcholine, bradykinin, thrombin, adenosine diphosphate, or calcium ionophores. Thus, it remains to be demonstrated that a single cell population bears the hallmarks of two different mechanisms for regulating nitric-oxide synthase or of two different nitric-oxide synthases. To our knowledge, there is no sound basis for the assertion that endothelial cells are the main source of nitrogen oxides in vivo, especially when one considers the potential contribution of the liver (11).

Finally, the introduction should not be misconstrued as suggesting that all the agents shown to induce endothelial cell nitrogen oxide release also induce a hypotensive vascular leak syndrome. In fact, the principal agent effective in this study, interferon-γ (IFN-γ), does not produce this toxic effect, even though it almost certainly causes the production of one or more of the other cytokines that synergize with IFN-γ in vitro to induce nitrogen oxide synthesis.

A very recent, important observation appears to go hand in hand with the findings of Kilbourn and Belloni—profound hypotension induced by injection of tumor necrosis factor in dogs was reversed by administration of NG-monomethyl-l-arginine and restored with l-arginine (34). However, two dissimilarities have not been explained. Hypotension began 20 minutes after injection of tumour necrosis factor without endothoxin (34), while endothelial cells only began to release nitrogen oxides 8 hours after exposure to cytokines, and only in the presence of endotoxin. This, together with the discrepancy between the in vitro observations reported by Kilbourn and Belloni and clinical experience with IFN-γ, emphasizes that mouse brain endothelial cell cultures model only some aspects of the processes leading to cytokine-induced hypotension in humans.

Nonetheless, the reports by Kilbourn and Belloni (33) and by Kilbourn et al. (34) provide a strong impetus for further studies of the inhibition of nitric-oxide synthases in experimental animals (35,36) and humans (37), with both substrate analogues (13,17) and other kinds of inhibitors (9). 1

References

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