Arginine Metabolic Enzymes, Nitric Oxide and Infection¹,²

Masataka Mori³ and Tomomi Gotoh

Department of Molecular Genetics, Graduate School of Medical Sciences, Kumamoto University, Kumamoto 860-8556, Japan

ABSTRACT Nitric oxide (NO) is synthesized from arginine by NO synthase (NOS), and the availability of arginine is one of the rate-limiting factors in cellular NO production. Citrulline that is formed as a by-product of the NOS reaction can be recycled to arginine by successive actions of argininosuccinate synthetase (AS) and argininosuccinate lyase (AL), forming the citrulline-NO cycle. AS and sometimes AL have been shown to be coinduced with inducible NOS (iNOS) in various cell types including activated macrophages, microglia, vascular smooth muscle cells, glial cells, neuronal PC12 cells, retinal pigment epithelial cells, and pancreatic β-cells. Coinduction of endothelial NOS (eNOS), AS, and AL are observed in human umbilical vein endothelial cells. In contrast, arginase can downregulate NO production by decreasing intracellular arginine concentrations. iNOS and arginase activities are regulated reciprocally in macrophages by cytokines, and this may guarantee the efficient production of NO. In contrast, iNOS and arginase isoforms (type I and/or II) are coinduced in immunostimulated macrophages, but not in PC12 cells and glial cells. These results indicate that NO production is modulated by the recycling and degradation of arginine. Arginase also plays an important role in regulation of polyamine and proline synthesis. J. Nutr. 134: 2820S–2825S, 2004.

KEY WORDS: • arginine • arginase • argininosuccinate synthetase • nitric oxide • nitric oxide synthase

Nitric oxide (NO)⁴ is a messenger molecule functioning in vascular regulation, host immune defense, neurotransmission, and other systems (1). Diseases such as vascular dysfunctions are associated with the impaired production of NO, whereas septic shock, cerebral infarction, diabetes mellitus, and neurodegenerative disorders are associated with NO overproduction (1). NO is synthesized from arginine by the 3 NO synthase (NOS) isoforms, neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). The availability of intracellular arginine is a rate-limiting factor in NO production. In this review, regulation of NO production by arginine metabolic enzymes in macrophages and other cell types is discussed.

Metabolism of arginine and NO

Arginine is a precursor for synthesis of urea, polyamines, creatine phosphate, and NO (Fig. 1). It is transported from blood into cells by cationic amino acid transporter (CAT) isoforms. Arginine is synthesized from citrulline by successive actions of argininosuccinate synthetase (AS) and argininosuccinate lyase (AL), the third and fourth enzymes of the urea cycle (ornithine cycle). The major site of arginine metabolism in ureotelic animals is the liver, where arginine generated in the urea cycle is rapidly converted to urea and ornithine by arginase with no net synthesis of arginine. Another major site is the kidney, where arginine is synthesized from citrulline and is released into the blood. However, many other tissues and cell types also contain low levels of AS and AL. In adult animals, citrulline is produced primarily by the small intestine from NH₃, CO₂, and ornithine by carbamylphosphate synthetase 1 and ornithine transcarbamylase, the first 2 enzymes of the urea cycle, and is supplied to the kidney and probably to other tissues for synthesis of arginine. Citrulline is also formed from arginine as a co-product of the NOS reaction, and this citrulline may be recycled to arginine if AS and AL are present in the same cell, forming the citrulline-NO cycle. In contrast, arginine is hydrolyzed to urea and ornithine by arginase. Thus, arginase and NOS use arginine as a common substrate and may compete with each other for this substrate.

Citrulline-NO cycle in iNOS-expressing cells

Citrulline, a by-product of the NOS reaction, can be recycled to arginine by AS and AL in the citrulline-NO cycle. AS and AL are expressed strongly in the liver and kidney and at very low levels in many other tissues and cells. The citrulline-
NO cycle was first shown to function in bovine aorta endothelial cells (2) and rat peritoneal macrophages (3). When iNOS is induced in various cells stimulated by bacterial lipopolysaccharide (LPS) and cytokines, AS and sometimes AL are coinduced. Expression of iNOS, AS, and AL in unstimulated and stimulated tissues and cells is summarized in Table 1. iNOS and AS were first found to be coinduced in murine macrophage-like RAW 264.7 cells after stimulation with LPS and interferon-γ (IFN-γ) (7), cultured rat aortic smooth muscle cells stimulated by LPS and IFN-γ (10), and cultured rat and human pancreatic β-cells treated with cytokines (17). AS was not induced in these cells, probably reflecting that AS, not AL, is rate-limiting in the citrulline-arginine recycling reaction in many cells. Coinduction of iNOS and AS has also been shown in activated human tumor cell lines (20), rat glioma C6 cells (12,28), mouse microglial cells (10), and rat retinal pigment epithelial cells (16). NO was produced from citrulline as well as from arginine in activated C6 cells and retinal pigment epithelial cells, indicating that the citrulline-NO cycle is actually functioning in these cells (12,16). When rat astroglia-rich primary cultures were treated with LPS and IFN-γ, AS was induced and colocalization of AS and iNOS was shown in microglial cells (13). Surprisingly, when nerve growth factor-factor differentiated rat neuronal PC12 cells were immunostimulated, iNOS and AS were highly coinduced, and a large amount of NO was produced from citrulline and from arginine (14). The significance of this NO production in this neuronal cell remains to be studied. Coinduction of iNOS and AS has also been found in vivo in the lung, heart, liver, spleen, skeletal muscle, testis, and elsewhere by the administration of LPS into rats (23) and mice (6). Immunoblot analysis showed that macrophages are strongly positive for both iNOS and AS after LPS treatment (23). Coinduction of iNOS and AS was shown in an animal model of endotoxin-induced uveitis (27). When rats were subjected to intervitreal injection of LPS, iNOS and AS were induced in infiltrating inflammatory cells in the vitreous, iris, ciliary body, and inner layers of retina. Coinduction of iNOS and AS was also observed in an animal model of fungal infection (24). When mouse lungs were exposed to filamentous fungus Fusarium kyushuense, granuloma appeared in the lung and iNOS and AS were highly coinduced. These results suggest that the citrulline-NO cycle is operating in vivo in various tissues and cells under inflammation. Regulation of the citrulline-NO cycle in macrophages and other iNOS-expressing cells in early and late stages of infection is schematically shown in Figure 2.

### Table 1

<table>
<thead>
<tr>
<th>Cells and tissues</th>
<th>NOS</th>
<th>Arginine recycling</th>
<th>Arginase</th>
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<tr>
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<td>iNOS: +</td>
<td>AS: +</td>
<td>I: +</td>
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<td>(mouse)</td>
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<td>AL: +</td>
<td>II: +</td>
<td>(5,6)</td>
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<tr>
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<td>AS: +</td>
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- , not expressed under normal conditions; +, expressed; ++, highly expressed; −, not induced by LPS, cytokines, etc.; ±, weakly induced; (+), induced.
Citrulline-NO cycle in nNOS-expressing cells

iNOS is induced in the brain astrocytes by systemic treatment of rats with LPS. We found that eNOS is induced in the rat brain by intraperitoneal injection of LPS of a smaller amount than that required for induction of iNOS (21). eNOS was located in astrocytes of both gray and white matters as well as in blood vessels. This induction of eNOS may be involved in early pathophysiologic response against systemic infection before iNOS is induced with progression of the infection. Coinduction of AS with eNOS remains to be tested.

Decreased availability of arginine and impaired production of NO have been implicated in the development of endothelial dysfunction. However, we found that eNOS, AS, and AL are coinduced in the aorta in early stages of streptozotocin-induced diabetic rats and that NO production is increased (11). Coinduction was also observed in human umbilical vein endothelial cells by transforming growth factor (TGF)-β1. These results suggest that TGF-β1 works antiatherogenically at early stages of diabetes by increasing NO production, whereas prolonged elevation of TGF-β1 functions atherogenically by inhibiting endothelial cell growth. Quite recently, AS and AL were shown to colocalize with eNOS in calveolae, a subcompartment of the plasma membrane (29). This suggests that the eNOS-AS-AL complex in calveolae plays a key role in endothelial NO production.

Citrulline-NO cycle in eNOS-expressing cells

NO plays an important role in enteric transmission. The capacity of NO-releasing enteric nerves to recycle citrulline to arginine and thereby sustain enteric neurotransmission was shown (30). AS and AL were both localized in nNOS-expressing myenteric and submucosal neurons in the canine proximal colon. Enteric inhibitory nerve transmission was inhibited by arginine analogues and this inhibition was restored by excess citrulline as well as by excess arginine. Colocalization of AS and AL with nNOS was also reported in neuronal cell bodies in the porcine sphenopalatine ganglia (31), in neurons and nerve fibers in the myenteric plexus of canine gastrointestinal tract (32), and in neurons in rat gastric fundus (33). With the use of in vitro tissue bath techniques, citrulline was shown to reversibly inhibit or induce nitric oxide synthase activity in isolated porcine pial veins produced by NOS inhibitors (31). In longitudinal smooth muscle strips of rat gastric fundus, citrulline as well as arginine were capable of preventing NOS inhibitor-induced inhibition of electrically induced nitrergic relaxation (32). These results indicate that recycling of citrulline is important in providing sufficient amounts of nNOS substrate in gastrointestinal functions.

Regulation of arginase and NO synthesis

Both NOS and arginase use arginine as a common substrate and arginase may downregulate NO production by competing with NOS for arginine. Arginase activity increased in mouse peritoneal macrophages after exposure to LPS (34). Wound and peritoneal macrophages convert arginine to citrulline and ornithine at comparable rates, indicating that both NOS and arginase pathways are functioning (35,36). In mouse bone marrow-derived macrophages, NOS and arginase activities are regulated reciprocally by Th1 and Th2 cytokines, events that may guarantee the efficient production of NO (8). Differential expression of iNOS and arginase in RAW 264.7 macrophages (37) and mouse peritoneal macrophages (38) was also reported.

Arginase exists in 2 isoforms, liver-type arginase I (39,40) and nonhepatic type arginase II (9,41,42). Arginase I is located in the cytosol, whereas arginase II is located in the mitochondrial matrix. Expression of arginase isoforms in tissues and cells is summarized in Table 1. iNOS and arginase II are coinduced in LPS-stimulated RAW 264.7 macrophages (9,43). Surprisingly, however, we found that arginase I, not arginase II, is coinduced with INOS in rat peritoneal macrophages and in vivo in rat lung after LPS treatment (4). Induction of arginase I is mediated at least in part by the preceding induction of CAAT/enhancer binding protein (C/EBP)β, a transactivator of the arginase I gene (4,44). In contrast, both arginase I and arginase II as well as iNOS are induced in LPS-activated mouse peritoneal macrophages (5,6); arginase II is induced early, while arginase I is induced much later. Since overproduction of NO is toxic to macrophages and neighboring cells, a mechanism to prevent overproduction of NO may exist. We speculate that arginase I is induced in the late stage of endotoxemia and prevents sustained overproduction of NO. Ochoa et al. (45) reported that arginase I is markedly induced in human mononuclear cells after injury, with a concomitant decrease of plasma concentrations of arginine and NO metabolites. In contrast, Kapka-Lenhart et al. (46) reported that endogenous levels of arginase...
I am limiting for polyamine synthesis, but not for NO synthesis, in activated RAW 264.7 macrophages. Interestingly, induced arginase I in macrophages can enhance tumor cell growth by providing them with polyamines and can suppress tumor cytotoxicity by reducing NO production (47). In contrast, arginase II is induced early and may provide ornithine for polyamine and/or proline (and thus collagen) synthesis. Regulation of arginase isoforms in macrophages in early and late stages of infection is shown schematically in Figure 2.

\(^N\)\(^2\)-Hydroxy-l-arginine (NOHA), an intermediate in the biosynthesis of NO, is a potent competitive inhibitor of arginase I (48,49). Substantial amounts of this intermediate were found to be released in the medium of LPS-treated rat alveolar macrophages (50), and the NOHA inhibition of arginase may ensure sufficient availability of arginine for high-output production of NO in activated macrophages. When RAW 264.7 cells are exposed to LPS and IFN-\(\gamma\), iNOS is induced and NO production increases. When dexamethasone and dibutyryl cAMP that added, both iNOS and arginase II are induced and NO production is much decreased (51). This means that induced arginase II downregulates NO production by depleting intracellular arginine. Downregulation of NO production by arginase was noted in the case of NO-mediated apoptosis. When RAW 264.7 cells are treated with LPS and IFN-\(\gamma\), the cells undergo NO-dependent apoptosis. This apoptosis was prevented when arginase II was induced or expressed by cDNA transfection (51). Arginase I was also effective in preventing apoptosis. Thus both cytosolic arginase I and mitochondrial arginase II are effective in downregulating NO production and in preventing NO-mediated apoptosis in activated macrophages. However, NOHA appears to work differently in vascular smooth muscle cells. Ignarro et al. (52) showed that increasing arginase I activity in rat aortic smooth muscle cells results in increased polyamine production and cell proliferation, and that NOHA and NO may interfere with cell proliferation by inhibiting arginase and ornithine decarboxylase, respectively.

Vascular endothelial cells express iNOS and produce NO on stimulation with LPS and cytokines. Rabbit aortic endothelial cells contain both arginase isoforms, and LPS induced both iNOS and arginase II, but not arginase I (53). Induction of iNOS by LPS and cytokines resulted in production of large quantities of NO, citrulline, and NOHA, but urea production was markedly diminished, probably due to the NOHA inhibition of arginase. Li et al. (54) reported that arginase can modulate NO synthesis in bovine venular endothelial cells and that basal levels of arginase I and II are limiting for endothelial synthesis of polyamines, proline, and glutamate. Hein et al. (55) studied the mechanism of the impairment of NO-dependent vasodilation by ischemia-reperfusion and found that arginase activity is increased in arteries of porcine heart after treatment and that NO production is decreased. An arginase inhibitor enhanced NO production and dilation in normal vessels and also restored the NO-mediated dilation after ischemia-reperfusion. Berkowitz et al. (56) reported that arginase decreases NO production in aortic rings from rats and that arginase upregulation contributes to endothelial dysfunction of aging blood vessels. Therefore, arginase may have important implications for cardiovascular function, wound healing, and angiogenesis by modulating synthesis of NO, polyamines, proline, and glutamate.

NO is involved in physiological and pathological processes in the brain. Possible regulation of NO production by arginase in the brain is of great interest. RNA blot analysis showed that arginase II is expressed at low levels in various portions of the human brain, whereas arginase I expression was barely detectable (57). In situ hybridization of the rat brain showed that nNOS mRNA is restricted to a subpopulation of neurons, whereas arginase II mRNA is present ubiquitously in glial cells and neurons, including the nNOS-positive neurons (22). Therefore, in the neurons expressing both nNOS and arginase II, arginase II may regulate nNOS-dependent NO production. However, neither arginase I nor arginase II was detectable in control and activated neuronal PC12 cells (14). In endotoxin-induced uveitis rats, arginase I was coinduced with iNOS in infiltrated inflammatory cells in the eyes (27), and may regulate NO production in the eyes. In contrast, both arginase I and II are highly expressed in rat retinal pigment epithelial cells (16). When cells are immunostimulated, iNOS is markedly induced, whereas arginase I and II remain little changed. The roles of arginase isoforms in this cell type remain to be studied.

**Therapeutic implications**

Various diseases are associated with the impaired production or overproduction of NO. Because arginine is one of the regulating factors of NO synthesis, it is theoretically feasible to regulate NO production by controlling the availability of arginine for NOS reaction. To prevent the overproduction of NO in septic shock, NOS inhibitors are used clinically. In addition, the inhibition of arginine recycling, especially at the step of AS, is expected to be effective. Forced induction of arginase may also be effective. Excess NO is implicated in the destruction of \(\beta\)-cells leading to the development of type 1 diabetes. NO production in \(\beta\)-cells is regulated positively by the citrulline-NO cycle (17) and negatively by arginase (18). Therefore, modulation of either pathway may be effective in attenuating NO production. Chlamydia causes numerous chronic diseases. Huang et al. (58) reported that the quantity of NO release by macrophages positively regulates Chlamydia-induced disease, and suggested that different susceptibility of different mouse strains to Chlamydia is attributable to arginase II activity. In conditions associated with impaired NO production such as many vascular diseases, administration of arginine is sometimes effective. In addition, activation or induction of arginine recycling or inhibition of arginase will also be useful. Impaired production of NO in alveolar macrophages is implicated in airway hyperresponsiveness. Klausen et al. (59) showed that glucocorticoids inhibit LPS-induced up-regulation of arginase activity in rat alveolar macrophages, and may contribute to the beneficial effects of corticoids in the treatment of inflammatory airway diseases. Carriliza and Moncada (19) found that arginase II expression is upregulated in synovial fluid cells of rheumatoid arthritis and may down-regulate NO synthesis and provide ornithine for polyamine synthesis. Impaired NO production can be a crucial factor in abnormal proliferation of keratinocytes in psoriasis. Bruch-Gerharz et al. (60) showed that arginase I is highly coincided with iNOS in the hyperproliferative psoriatic epidermis and competes with iNOS for arginine. Thus, arginase inhibition may be effective in preventing keratinocyte proliferation. Taking together, arginine metabolic enzymes are pertinent therapeutic targets to control NO production in various diseases associated with disordered NO production.

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**LITERATURE CITED**


