Hypothesis

Endogenous nitric oxide synthase inhibitors are responsible for the L-arginine paradox

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Abstract L-Arginine, the substrate of nitric oxide (NO) synthases (NOSs), is found in the mammalian organism at concentrations by far exceeding K_M values of these enzymes. Therefore, additional L-arginine should not enhance NO formation. In vivo, however, increasing L-arginine concentration in plasma has been shown repeatedly to increase NO production. This phenomenon has been named the L-arginine paradox; it has found no satisfactory explanation so far. In the present work, evidence for the hypothesis that the endogenous NOS inhibitors methylarginines, asymmetric dimethylarginine being the most powerful (IC_{50} 1.5 μM), are responsible for the L-arginine paradox is presented.

Key words: L-Arginine paradox; Nitric oxide synthase; Endogenous inhibitor; Methylarginine; Asymmetric dimethylarginine

1. Introduction

L-Arginine is the exclusive physiological substrate for various isoforms of the nitric oxide (NO) synthases (NOSs) family (EC 1.14.13.39) which catalyzes the oxidation of L-arginine to NO and L-citrulline [1]. At least three isozymes have been identified for NO production from L-arginine [2]. These isozymes have been classified as isoform I in neuronal (nNOS) and epithelial cells, isoform II in cytokine-induced cells (iNOS) and isoform III in endothelial cells (eNOS). nNOS and eNOS are dependent on and iNOS is independent of Ca^{2+} [2]. Half-saturating L-arginine concentrations (K_M) were reported as 1.4–2.2 μM for nNOS, 2.8–32.3 μM for iNOS and 2.9 μM for eNOS [2]. L-Arginine is supplied to cells by a y^+ transport system specific for cationic amino acids [3]. Freshly isolated endothelial cells have been found to contain up to 2 mM L-arginine [4]. Considering this and a K_M of 2.9 μM for L-arginine for eNOS [2], this enzyme should be saturated in endothelial cells. It is surprising that intravenous (i.v.) or oral supplementation of L-arginine in vivo in humans augments endothelial NO production [5–18]. Supplementation of L-arginine to hypercholesterolemic animals [5,6] and humans [7,8], in which endothelium-dependent vasodilatation is impaired, was found to improve endothelial dysfunction by increasing NO production [5–8]. Also, supplementation of L-arginine. This phenomenon, generally known as the L-arginine paradox, has found no satisfactory explanation so far. We here present evidence that concentrations of endogenous NOS inhibitors in vivo provide a satisfactory explanation for this phenomenon.

2. Proposal

The L-arginine paradox may be solved by proposing that NOS isoforms are potently inhibited in vivo and in vitro in intact cells by endogenously produced compounds. Mechanism of inhibition, inhibitor potency and intracellular concentrations of inhibitors, L-arginine and cofactors including Ca^{2+} regulate NOS activity and consequently NO production in cells capable of synthesizing NOS. Under physiological conditions, the enzyme activities of NOS isoforms are lowered to a fraction of their maximum activities (V_{max}) although the enzymes are exposed to concentrations of L-arginine which theoretically should allow the enzymes to operate at the V_{max} values of the uninhibited enzymes. Under pathological conditions, increased intracellular concentrations of the inhibitors cause additional decreases of the activity of the NOS enzymes which result in NO formation rates below the physiological levels. Under physiological and pathophysiological conditions, i.v. or oral administration of L-arginine causes an increase in circulatingL-arginine concentrations which leads to an increase in intracellular L-arginine concentrations and exchange of intracellular inhibitors against extracellular L-arginine via the y^+ transport system in NOS producing cells. Antagonization of L-arginine with competitive inhibitors and decrease of intracellular concentrations of competitive and non-competitive inhibitors by extracellular L-arginine cause an increase in NOS activity and augmentation of NO production. These effects are dependent on the administered amount of L-arginine and last as long as circulating L-arginine concentrations are above the L-arginine concentrations before administration.

3. Evidence supporting this hypothesis

1. Presently, two endogenous potent inhibitors of NOS-catalyzed formation of NO from L-arginine are known, i.e. the methylated L-arginines, N^G,N^G-dimethyl-L-arginine (asymmet-

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Abbreviations: NO, nitric oxide; NOS, nitric oxide synthase; ADMA, asymmetric dimethylarginine; NMA, (mono)methylarginine; SDMA, symmetric dimethylarginine

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ric dimethylarginine, ADMA) and N^G-methyl-L-arginine (NMA) [18–27]. Plasma concentrations of ADMA in healthy humans are in the range of 0.5–1.0 μM [23–27]. ADMA and NMA occur in endothelial cells of rabbits at a concentration of 5 μM each [28].

2. ADMA and NMA are potent inhibitors of various NOS isoforms (Fig. 1) [19,29–34]. ADMA and NMA inhibit NO synthesis with comparable potencies in vitro and in vivo, in blood vessels and macrophages, in animals and in man [19]. ADMA is a non-competitive inhibitor (K_i 0.4 μM; K_{ii} 1.6 μM; IC_{50} 1.5 μM) of an isolated nNOS (Fig. 1) [34]. The IC_{50} for ADMA in rat brain homogenates is 2 μM [35]. NMA is not simply a competitive inhibitor of several NOS isoforms (K_i 3.9, 0.65 and 0.7 μM for iNOS, nNOS and eNOS, respectively [36]), but it also induces time-dependent inactivation of macrophage and brain NOS [29–36]. ADMA is a more potent inhibitor of nNOS than NMA (Fig. 1) [33].

3. In various pathological conditions associated with endothelial dysfunction such as essential hypertension, atherosclerosis and hypercholesterolemia, ADMA plasma concentrations are elevated while urinary excretion of the NO metabolites nitrite and nitrate (Fig. 2) and of cGMP, the second messenger of NO, are reduced both in animals and in humans; under these conditions, the L-arginine concentration is almost normal [24,26,27,37–39].

4. I.v. or oral administration of L-arginine to healthy and ill humans as well as to animals results in endothelium-dependent vasodilatation, in increase of plasma nitrite concentration and enhanced urinary excretion of nitrate (Fig. 2) and cGMP [9–11,14]. These effects correlate with plasma L-arginine concentrations and last as long as the L-arginine plasma concentration is several fold higher than the respective basal level [9,10]. Significant endothelium-dependent, L-arginine-induced vasodilation, NO and cGMP production occurs after i.v. infusion of 30 g of L-arginine in healthy humans that results in plasma L-arginine concentrations 10–80-fold higher than the basal levels (Fig. 2) [9–11,14]. The extent of conversion of L-[guanidino-$^{15}N_2$]arginine to [^{15}N]nitrate in cholesterol-fed rabbits strongly and inversely correlates ($r = 0.77$, $P < 0.05$) with ADMA plasma concentrations [40]. The results from a meta-analysis of currently available literature data on NO production in dependence on plasma L-arginine to AMDA ratio in humans and animals show a good correlation between these parameters (Fig. 2) and strongly support our hypothesis.

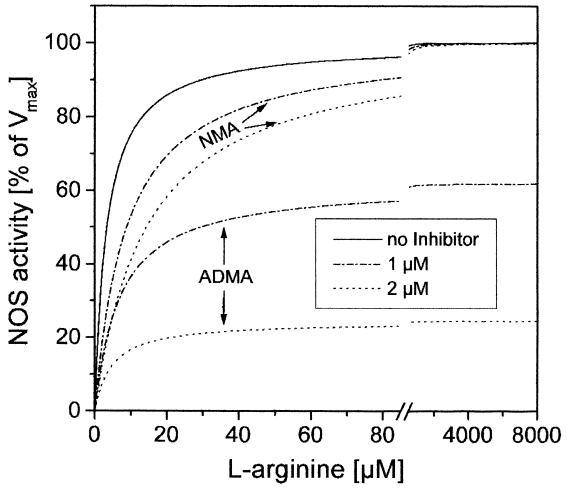

Fig. 1. Simulation of nNOS activity for competitive (by NMA; K_i 0.6 μM [32]) and non-competitive (by ADMA; K_i 0.4 μM; K_{ii} 1.6 μM [33,34]) mechanisms-based inhibition at inhibitor concentrations of 0, 1 and 2 μM. K_M was set to 3.1 μM [33]. $V_{max} = 0.204$ μmol [^{15}N]nitrite per min per mg nNOS was set to 100% for the non-inhibited enzyme [33]. L-Arginine concentration was varied as indicated.

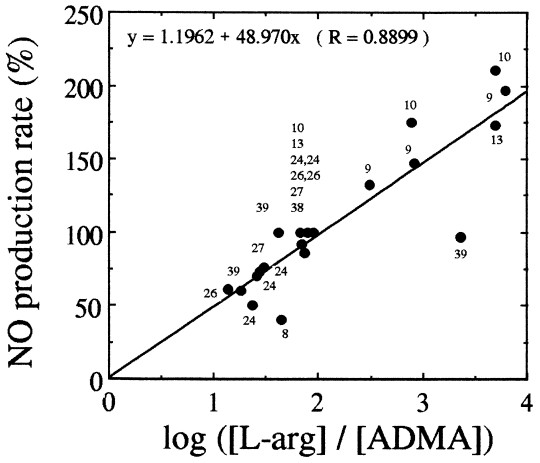

Fig. 2. Meta-analysis between NO production in vivo in humans and animals and logarithmic (log) plasma L-arginine to ADMA concentration ratio. Data were involved in this meta-analysis from studies described in [9,10,13,24,26,27,38,39]. Symbols are accompanied with the respective reference numbers. NO production of 100% is defined as the plasma nitrite concentration or urinary nitrate excretion in the respective control group with a log ([L-Arg]/[ADMA]) value of about 1.9. NO production values above 100% and below 100% indicate increased and decreased NO production, respectively, with respect to that of the control group.

5. ADMA, symmetric dimethylarginine (SDMA) and NMA compete with L-arginine transport mediated by the inducible human y^+ transport system hCAT-2B [3]. The inhibition of hCAT-2B-mediated L-arginine transport by SDMA is reversed by excess of L-arginine. Thus, the inhibitory effect of these methylarginines on NOS isozymes in various intact cells may be affected by the extracellular concentration of ADMA, SDMA, NMA and L-arginine. Increased NO production seen in vivo after administration of L-arginine at high doses could result from an exchange of intracellular inhibitors against circulating L-arginine.

4. Conclusions and future prospects

The evidence cited above can provide a satisfactory explanation for the L-arginine paradox by assigning ADMA, the most powerful endogenous inhibitor of NOS, a central role. However, few data exist on the transport of ADMA in and out of NO producing cells and additional work is required to fully characterize its intracellular concentrations. Further, the effect of L-arginine and ADMA on NOS cofactor requirement has not been evaluated. Data from such investigations would help to prove the present hypothesis.

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