L-Arginine Metabolic Pathways

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Abstract: The present review will give an overview of the biochemistry and metabolism of L-arginine focusing on the mechanisms which may either provide or limit cellular availability of this amino acid for nitric oxide synthases and arginase / polyamine pathway and describe possible interactions between these pathways. In addition, emerging species differences in the expression and role of arginases are addressed, as they may have considerable impact on the translation of results from animal models into human. In addition, the recently described G-protein-coupled receptor GPRC6A receptors as target for L-arginine and drugs modulating the activity of arginases and nitric oxide synthases are discussed.

Keywords: Arginase, CAT - cationic amino acid transporter, collagen synthesis, nitric oxide synthesis, polyamines, GCPRC6, L-arginine, L-ornithine, L-proline.

1. INTROCUTION

Since the first isolation of L-arginine in 1886 [1], the biochemistry and physiology of this amino acid has been a field of active research. However, the discovery about 100 years later, that L-arginine is the only physiologically significant substrate for the synthesis of nitric oxide (NO) [2-6], has markedly stimulated the interest in the complex metabolism of this amino acid, as NO has been identified as an important intra- and transcellular signalling molecule involved in the regulation of many physiological and pathophysiological processes in the mammalian organism [for reviews 7-14].

The present review will give an overview of the biochemistry and metabolism of L-arginine focusing on the mechanisms which may either provide or limit cellular availability of this amino acid and possible interactions between the different pathways. In addition, emerging species differences are addressed, as they may have considerable impact on the translation of results from animal models into human.

2. BIOSYNTHESIS OF L-ARGININE

L-Arginine has been characterized as semi-essential amino acid, in that it is non-essential in the healthy adult organism of most mammals, but has to be supplemented in the growing organism, after trauma or during various disease states [15-19]. Normal L-arginine plasma levels are in the range of 100 – 200 μM [20-23]. It is important to note that even in adult mammalian organisms not all of the enzymes required for *de novo* synthesis of L-arginine are expressed in every tissue. Three steps in the L-arginine biosynthesis, differentially compartmented in the mammalian organism may be discriminated: Biosynthesis of I) L-ornithine, II) L-citrulline and III) L-arginine.

I) The biosynthesis of L-ornithine form food- or blood-derived L-glutamine and L-proline occurs almost exclusively in the small intestine [24,25] as the L-Δ^1-pyrroline-5-carboxylate (P5C) synthetase (P5CS) (one of the enzyme required for the conversion of L-glutamine into L-ornithine, see Fig. **1**) is exclusively located in the intestinal mucosa [26,27]. Likewise, proline dehydrohenase (PROHD), which catalyses the formation of P5C from proline (Fig. **1**), is also mainly expressed in the intestinal mucosa, although some activity is also detected in the liver and kidney [26,27]. As outlined in more detail below L-ornithine is also produced by the arginases using L-arginine as substrate (Fig. **1**), but this reaction may be considered as L-arginine consuming rather than as initial step in the L-arginine biosynthesis.

II) The biosynthesis of L-citrulline from L-ornithine depends on the presence of ornithine carbamoyltransferase (OTC) and carbamoylphosphate synthetase 1 (CPS1). The expression of both enzymes is restricted to the mitochondrial matrix of hepatocytes and epithelial cells of the small and to a minor extent large intestine [28,29]. In the liver, this reaction is part of the urea cycle, whereas L-citrulline produced in the intestine is released into the circulation. Most of this circulating L-citrulline is taken up by cells of the proximal tubulus of the kidney converted into L-arginine and finally released into the circulation for the benefit of other cells [30-35].

III) The biosynthesis of L-arginine from L-citrulline is performed by the cytosolic enzymes argininosuccinate synthetase 1 (ASS1) and argininosuccinate lyase (ASL). The reaction catalysed by ASS requires L-aspartate as co-substrate and is the rate-limiting step. In contrast to the mitochondrial enzymes of the urea cycle OTC and CPS1, ASS1 and ASL appear to be expressed in many cells, although the degree of expression and the efficiency of this pathway appear to differ considerably between different cells [36-47]. Since during NO-synthesis L-arginine, via N^{ω}-OH-L-arginine as intermediate product, is converted to L-citrulline [48-50], the immediate use of L-citrulline for the re-synthesis of

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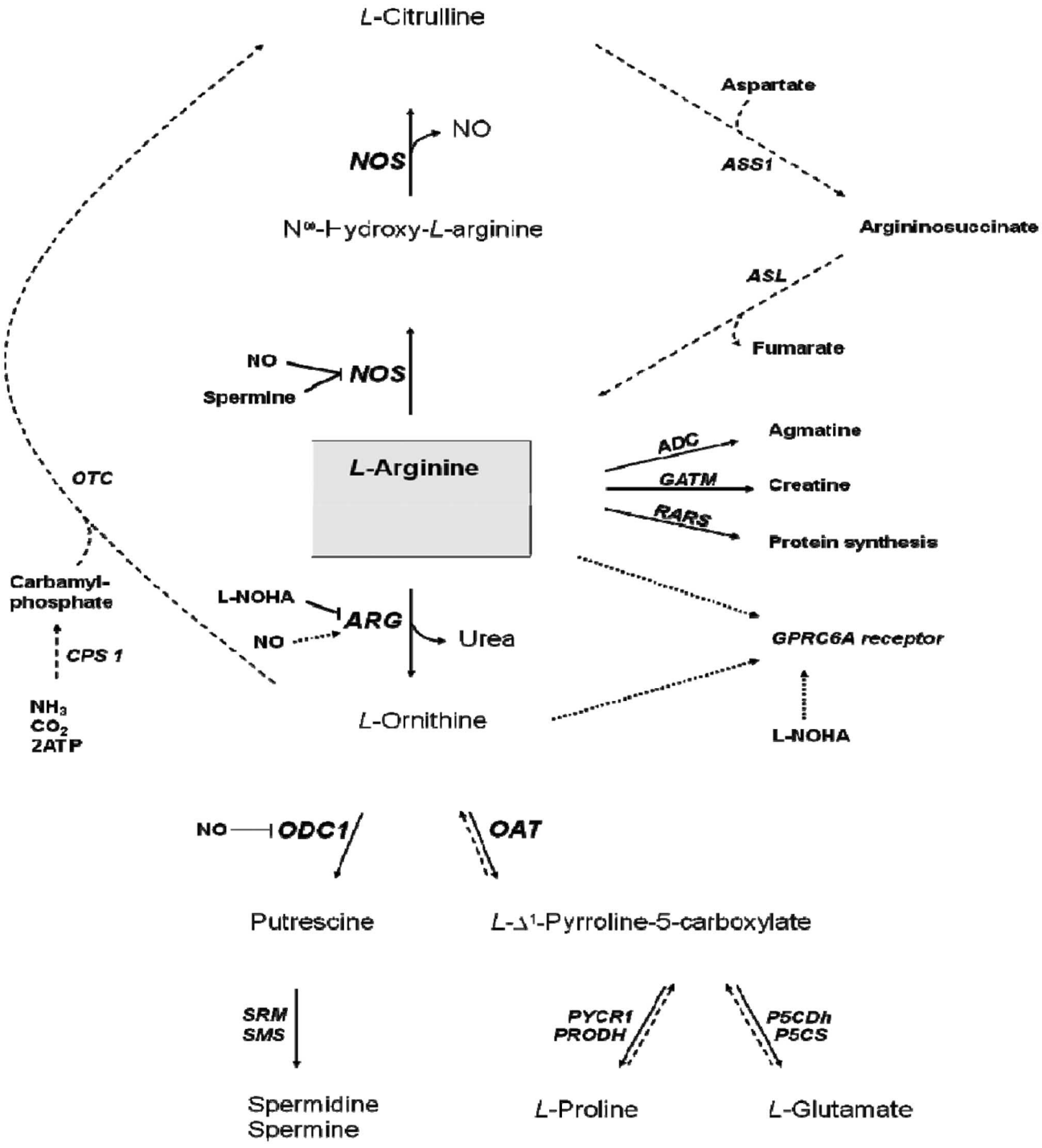

Fig. (1). Metabolic pathways of L-arginine.

ARG, arginase (EC 3.5.3.1); ASL, argininosuccinat lyase (EC 4.3.2.1); ASS1, argininosuccinat synthetase 1 (EC 6.3.4.5); OTC, ornithine carbamoyltransferase (EC 2.1.3.3); CPS1, carbamoyl-phosphate synthetase 1, mitochondrial (EC 6.3.4.16); ADC, arginine decarboxylase (EC 4.1.1.19); GATM, glycine aminotransferase (EC 2.1.4.1); RARS arginyl-tRNA-synthetase (EC 6.1.1.19); NOS, nitric oxide synthase (EC 1.14.13.39) ; ODC1, ornithine decarboxylase (EC 4.1.1.17); OAT, ornithine aminotransferase (EC 2.6.1.13); PYCR1, pyrroline-5-carboxylate reductase 1 (EC 1.5.1.2); P5CDh = ALDH4A1, aldehyde dehydrogenase familiy 4, member A1 (EC 1.5.1.12); P5CS, pyrroline-5-carboxylase synthetase (= ALDH18A1, EC 1.2.1.41); PRODH, proline dehydrogenase (oxidase) 1 (EC 1.5.99.8); SRM, spermidine synthase (EC 2.5.1.16); SMS, spermine synthase (EC 2.5.1.22).

L-arginine may be an effective way to ensure sufficient substrate supply for a prolonged NO synthesis and has therefore been described as citrulline/NO cycle [see 27,51]. The functional significance of this L-arginine providing pathway for the synthesis of NO is further supported by observations showing that ASS1 and ASL are co-localized with endothelial NOS in caveoli [52] and that stimuli which induce a prolonged NO synthesis by induction of the inducible NOS (iNOS, see also below) can also cause an up-regulation of ASS1 [e.g. 40,41,43,45,53-56]. Nevertheless, despite the up-regulation of ASS1, the availability of L-arginine may remain rate limiting for the synthesis of NO and the cellular uptake of L-arginine may determine the amount of NO synthesised, as demonstrated for example in different macrophages [38,39,45,57] (see also below).

3. CELLULAR UPTAKE OF L-ARGININE

There are several mechanisms by which L-arginine may be transported into the cells. First, there is a family of specific cationic amino acid transporter (CAT) proteins consisting of four members (CAT-1, -2A, -2B and 3) with CAT-2A and 2B being splicing variants of the same gene. For detailed description of their structure and functions it is referred to a recent review of Closs *et al*. [58]. Here, only a short overview will be given. These CATs belong to the larger family of solute carriers 7 (SLC7) and therefore the names SLC7A1, SLC7A2 and SLC7A3 have been assigned for the genes of CAT-1, -2, and -3, respectively [59]. There is an additional homologous protein SLCA4 or CAT-4, which however lacks cationic amino acid transport activity [60] and other physiological functions have not yet been identified.

The different CAT proteins consist of between 619-658 amino acids and have been predicted on the basis of hydropathy analysis to have 14 transmembrane domains [61], a model supported by further experimental data [58]. All CATs (except of CAT-4) selectively transport (at physiological pH) cationic amino acids in a sodium-independent manner, but there are some differences particularly with regard to the affinity for amino acids. For CAT-1, K_m values for L-arginine, L-ornithine and L-lysine in range of 100 to 150 μM have been described [62,63]. Further functional characteristics, such as "trans-stimulation" (stimulation by substrate at the trans-side), indicated that CAT-1 is a molecular correlate of the previously described amino acid transport *system* y^+ [64,65]. CAT-2B and CAT-3 are also considered as *system* y^+-like transporters as they show similar transport characteristics, although with somewhat lower affinities for cationic amino acids. On the other hand, CAT-2A has clearly distinguished properties. In particular, it shows a substantial lower substrate affinity for cationic amino acids than the other CATs [63,66].

CAT-1 appears to be expressed in almost every tissue except the liver. Nevertheless, its expression levels appear to be modulated in a tissue and cell specific manner [e.g. 67-72]. CAT-2A is primarily expressed in the liver [63], but has been shown to be up regulated in skeletal muscle after surgical stress [73]. On the other hand, CAT-3, although abundantly expressed during embryonal development, appears to be restricted to brain tissue in the adult organism [74,75]. Most interestingly, pro-inflammatory mediators such as lipopolysaccharides (LPS) or interferon-γ (IFN-γ), which cause induction of the "high throughput" nitric oxide synthase (iNOS), an enzyme which consumes L-arginine to considerable extent [76], caused an up-regulation of cellular L-arginine uptake [e.g. 57,77-80], and this was associated with an up-regulation of CAT-2B [57,80,81]. Since iNOS-mediated NO synthesis in macrophages largely depends on cellular uptake of L-arginine [e.g. 39,45,82], the concomitant up-regulation of CAT-2B may help to ensure a sufficient substrate supply. The particular role of CAT-2B to provide sufficient substrate to iNOS is also supported by the observations that iNOS mediated NO synthesis in macrophages and astrocytes was markedly impaired after ablation of the CAT-2 gene [83,84]. Based on the observation that in rat macrophages iNOS inhibition by the specific inhibitor (AMT, 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine) resulted in reduced L-arginine uptake despite the increased expression of CAT-2B, a particular close link between iNOS and CAT-2B was suggested [81].

In addition to the specific CATs, several broad spectrum amino acid transport systems, system y^+L, $b^{0,+}$ and $B^{0,+}$, have been differentiated essentially on the basis of affinity and sodium-dependence of the transport of either neutral or cationic amino acids [64,65,85-87]. During the recent years it was possible to ascribe distinct molecular entities also to these different transporter systems, namely the heteromeric amino acid transporters (HATs) among them 4F2hc/y^+LAT1 and 4F2hc/ y^+LAT2 (SLC3A2/SLC7A7 and SLC7A6) and rBAT/$b0^{,+}$AT (SLC3A1/SLC7A9) [88-92].

In addition to changes in the expression levels of transporter proteins modulation of transporter activity may also play a role in the control of cellular L-arginine uptake. Major basic protein (MBP) polycationic peptide released from activated eosinophils [e.g. 93] has been described to contribute significantly to allergic airway hyper-responsiveness [94-96]. Polycationic peptides including MBP were shown to inhibit L-arginine uptake into rat alveolar macrophages and airway epithelial cells [79]. Moreover, exposure of alveolar macrophages to polycationic peptides resulted in reduced NO synthesis, most likely because of limited L-arginine availability [79]. The polyanion heparin which is able to act as antagonist against polycationic peptides [95] prevented the polycationic peptides mediated inhibition of L-arginine uptake [79]. In line with these observations are functional studies in which nitric oxide deficiency and airway hyper-reactivity of isolated tracheae from allergen-challenged guinea-pigs could be normalized by heparin application [97]. L-Ornithine released from cells with high arginase activity as for example activated macrophages [76,98] might be another endogenous modulator of L-arginine transport and thereby its intracellular availability. Since L-ornithine is a cationic amino acid and thus substrate of CATs it may competitively inhibit L-arginine uptake. Furthermore since CATs are trans-activated transporters, increased levels of extracellular L-ornithine may favour the extrusion of L-arginine and thereby additionally reduce intracellular L-arginine levels [58]. In isolated guinea-pig tracheae L-ornithine induced hyper-reactivity which might be caused by insufficient NO synthesis caused by L-arginine deficiency [99].

4. NITRIC OXIDE SYNTHASES

As the structure and function of NOSs have been reviewed extensively in the past years only a short overview and some selected features of these enzymes will be noted here. There are three NOS isoenzymes (NOS I = nNOS; NOS II = iNOS; NOS III = eNOS) [e.g.7,8,48,100-101] of which multiple splicing variants exist [e.g. 102-104]. These isoenzymes differ in structural properties, distribution, regulation and output of NO quantities. The NOSs are relatively large enzymes (135-160 kD) with two functional domains, the N-terminal catalytic domain which binds the heme prosthetic group and the redox cofactor tetrahydrobiopterin (H_4B) and the C-terminal reductase domain with the binding sites for FMN, FAD, and NADPH. All three NOSs are inactive in the monomeric form and dimerization through the heme domain is required for activity. All three NOS use L-arginine as substrate, and in a reaction, in which N^{ω}-hydroxy-L-arginine (L-NOHA) is an intermediate, NO and L-citrulline are released [e.g. 49,104-106] (Fig. **1**). The Km values for L-arginine of all three NOSs are in the lower micromolar range [49,100,105]. nNOS and eNOS are constitutively expressed in a variety of different cells and their activities are regulated by Ca^{+}-dependent calmodulin binding as well as by serin phosphorylation/dephosphorylation [104,107-112]. In contrast, iNOS is regulated only transcriptionally [8,101,113]. Induction can be elicited in a large variety of cells by bacterial toxins such as LPS and several pro-inflammatory cytokines such as interleukin 1-ß (IL-1ß), IFN-γ or tumor necrosis factor-α (TNF-α), whereas IL-4, IL-8, IL-10, transforming growth factor-ß (TGF-ß) and several other mediators can exert suppressive effects on iNOS gene transcription. Once expressed, iNOS is fully active independent of the cellular calcium levels because iNOS binds calmodulin already in the presence of low concentrations or even in the absence of calcium [114]. As a consequence, the induction of iNOS leads to a prolonged synthesis of large amounts of NO, a process which may essentially be limited by the availability of substrate and the life-time of the iNOS protein. Most interestingly, cellular L-arginine appears however not only to determine substrate availability for iNOS, but may also affect iNOS-mediated NO synthesis by stimulating translation of iNOS mRNA [115]. Therefore, mechanisms controlling cellular L-arginine availability (see also below) appear to be crucial for the regulation of iNOS-mediated NO synthesis. In this context it is interesting to note that particularly under conditions of limited L-arginine availability iNOS releases oxygen radicals which may directly interact with NO resulting in the formation of peroxynitrite [116], a highly reactive nitrogen species which has been claimed to be an important mediator for detrimental effects of iNOS [see 117]. Noteworthy too, during high throughput iNOS-mediated NO synthesis the intermediate product L-NOHA is released in amounts to be biologically active by its own [76] (see also below).

5. ARGINASES AND DOWN-STREAM, L-ORNITHINE-DEPENDEND PATHWAYS

Arginase, which catalyzes the hydrolysis of L-arginine to urea and L-ornithine, is a classical enzyme of the urea cycles, but is additionally expressed in many non-hepatic cells in which it may among others interfere with synthesis of NO (see below).

There exist two isoenzymes: arginase I was originally described as the hepatic and arginase II as the extrahepatic enzyme, but arginase I is also expressed in non-hepatic tissue and arginase II is found in the liver [27,118]. The two isoenzymes of arginase show a different subcellular distribution, arginase I has a cytosolic and arginase II a mitochondrial localization [119-121].

Two Mn^{2+} ions per arginase molecule are essential for maintaining the tertiary structure and full enzyme activity [122]. Furthermore, X-ray cristal structure analysis revealed a homotrimeric quaternary structure of arginase I as well as arginase II [123-126]. Although alterations in Mn^{2+} ions availability may affect enzyme activity [127] cellular arginase activity appears to be regulated essentially at the transcriptional level. Nonetheless, recent reports indicate that arginase activity may also be modulated by post-tanslational modification of the enzyme. Thus, it was shown that cysteine residues (C168 and C303) in arginase I can undergo S-nitrosylation and that in particular the modification of C303 causes stabilization of the arginase I trimer resulting in a sixfold increase in affinity for L-arginine [128]. By mechanisms not yet illuminated, uric acid was also shown to enhance arginase activity, again by increasing the affinity for L-arginine [129]. Uric acid may affect arginase I and II, as stimulatory effects were seen in endothelial cell, liver and kidney lysates.

Both arginase isoenzymes have an alkaline pH optimum, with maximal reaction velocities at pH 9.0–9.5. However, there appear to be some differences with regard to the enzymes kinetics between arginase I and II as well as between different species. Thus, Km values for L-arginine hydrolysis by human arginase I were 80 and 20 μM at pH 8.5 and 9.5, respectively [125], whereas the Km values exhibited by human arginase II at pH 7.5 and 9.5 were 0.3 and 5 mM, respectively [130]. For rat arginases the reported Km values are considerable higher (between 1-7 mM at pH 9.0-9.5 for the liver (arginase I) and 18 mM at pH 9.5 for the kidney (arginase II) enzyme [131-133].

Differences between arginase I and II appear also to exist with regard to their sensitivity towards inhibitors. L-Ornithine, the product of the arginase reaction is a relatively potent competitive inhibitor of rat arginase I, but a poor inhibitor of human arginases II (Table **1**). The *K*i for arginase I inhibition is comparable to the *Km* for L-arginine. This finding may support the idea that the primary function of arginase II may be net biosynthesis of L-ornithine [130]. Although L-NOHA, the intermediate product in NO synthesis, is a potent inhibitor of both arginase isoenzymes, its potency to inhibit human arginase II was about 20fold higher than its potency to inhibit rat arginase I (Table **1**). Similarly, the analogue L-nor-NOHA, the most potent arginase inhibitor described so far, was a more potent inhibitor of human arginase II than of rat arginase I (Table **1**). However, since comparable data of L-NOHA and L-nor-NOHA for human arginase I are not yet available it remains unclear at present whether these observations reflect differences in the sensitivity of isoenzymes or species differences, particularly as there are marked species differences with regard to the affinity of the substrate L-arginine (see above) and two other potent inhibitors of arginase, S-(2-

Table 1. Kinetics of Arginase Inibitors

	Arginase I		**Arginase II**	
	(rat liver arginase)	(human recombinant)	(human recombinant)	
	K_i (μM) (pH 7.4-7.5) or *K_d	K_d	K_i (μM) pH (7.4-7.5)	K_i (μM) (pH 9.5)
L-Ornithine	1000	n.a.	> 10.000 [5]	n.a.
L-NOHA	10-40 [1,2,3,4]	n.a.	1.6 [6]	2 [6]
L-nor-NOHA	0.1-0.5 [2,4]	n.a.	0.05 [6]	n.a.
BEC	2.2* [7]	0.27 [8]	0.31 [6]	0.03 [6]
ABH	0.1* [9]	0.005 [8]	0.25 [6]	0.009 [6]

Data from: [1] [134], [2] [135], [3] [136], [4] [137], [5] [130], [6] [138], [7] [139], [8] [125], [9] [140]; n.a., not available.

borono-ethyl)-L-cysteine (BEC) and 2(S)-amino-6-boronohexanoic acid (ABH) (Table **1**).

The expression of arginase isoenzymes appears to be regulated in a cell specific manner. Thus, in the rat corticosteroids up-regulate arginase I in the liver in a secondary manner [56,141], but inhibit the up-regulation of arginase I induced by LPS or granulocyte-macrophage colony-stimulating factor (GM-CSF) in macrophages [142,143] or by IL-4 and IL-13 in pulmonary fibroblasts [144]. LPS causes marked up-regulation of arginase I in rat macrophages, but not in pulmonary fibroblasts [Lindemann and Racké, unpublished observation], most likely because of the lack of expression of Toll-like receptor 4 in fibroblasts. On the other hand, the Th2 cytokines IL-4 and IL-13 cause up-regulation of arginase I in a number of different cells including macrophages [142], fibroblasts [144] and smooth muscle [145].

Marked species differences in the expression pattern of the arginase isoenzymes in non-hepatic cells have to be noted. Thus, in rat peritoneal [146,147] and alveolar [142] macrophages, both arginase isoforms can be detected, but arginase I appears to be the predominant enzyme. On the other hand, arginase I mRNA could not be detected in isolated human monocyte-derived macrophages [148] and human alveolar macrophages [Warnken and Racké, unpublished observations). In human alveolar macrophages only arginase II mRNA could be detected and the overall arginase activity was about 100fold lower than in rat macrophages [Warnken and Racké, unpublished observations]. Similarly, rat and mouse pulmonary fibroblasts express both arginase isoenzymes with arginase I being particularly sensitive to inductive stimuli [144,149], whereas in human lung fibroblasts only arginase II was detected [150,151]. Nonetheless, also in human arginase I is not restricted to liver, high levels of arginase I are expressed in granulocytes [148, 152] where it appears to be localized in the granules allowing release of the enzyme during activation. Thus, it appears that non-hepatic expression of arginase I is rather restricted to particular cells in human, but widespread distributed in rat and mouse.

L-Ornithine, the product of arginase, serves as substrate for ornithine decarboxylase (ODC), the key enzyme in the synthesis of polyamines which are important regulators of cellular growth and differentiation [153-155]. This, together with the fact that L-ornithine can also serve as precursor for the synthesis of L-proline [27] (Fig. **1**), an amino acid essential for the synthesis of collagen [156], favored the hypothesis that arginase might play an important role in tissue remodeling processes [e.g. 157-159]. This idea is supported by the observations that elevated arginase levels are found in tissues in which increased fibrotic remodeling processes occur such as fibrotic lung diseases (or respective animal models) [e.g. 149,160-162] or allergic asthma (or respective animal models) [e.g. 163-166]. In fact the synthesis of basal and TGF-β-induced collagen synthesis by mouse [149] and rat [150,151,167] pulmonary fibroblasts in culture was significantly reduced in presence of the arginase inhibitor L-NOHA. Strikingly however, collagen synthesis in human lung fibroblasts was not affected by two different arginase inhibitors (L-nor-NOHA and BEC) [150,151, and unpublished results]. Since human, in contrast to the rat, pulmonary fibroblasts express only arginase II (see above), these divergent functional observations demonstrate that the species difference in expression pattern determines species different functional roles of arginases in non-hepatic tissues. Furthermore, these observations suggest a role of arginase I rather than arginase II in the supply of L-ornithine for the biosynthesis of L-proline and collagen. On the other hand, arginase appears not to be crucially involved in the regulation of pulmonary fibroblasts proliferation. Although Th2 cytokines up-regulate the expression of arginase I and II and total enzyme activity in rat pulmonary fibroblasts [144], arginase inhibitors did affect neither basal nor IL-4- nor IL-13-induced proliferation [150,151,167]. Likewise, in rat human pulmonary fibroblasts arginase inhibitors did neither affect basal nor PDGF-induced proliferation, although PDGF caused a marked increase in arginase II expression in these cells [168,169]. On the other hand, the ODC inhibitor DL-α-difluoromethylornithine (DFMO) largely attenuated IL-4- and IL-13-induced proliferation of rat pulmonary fibroblasts [167], indicating that polyamines are crucially involved in the pro-proliferative effects of these cytokines, but arginase-

mediated supply of L-ornithine might not be a limiting factor in polyamine synthesis.

6. INTERACTIONS BETWEEN NITRIC OXIDE SYNTHASE AND ARGINASE-POLYAMINE PATHWAYS

NOSs and arginase both utilize L-arginine and there appear to be multiple interactions between both pathways. Evidence that arginase may limit L-arginine (i.e. substrate) availability for NO synthesis was first demonstrated in rat alveolar macrophages. Inhibition of arginase by the specific inhibitor, N^{ω}-hydroxy-D,L-indospicine [170] resulted in marked increase in L-arginine turnover by iNOS [171]. A similar shift of L-arginine utilization into the NOS pathway after inhibition of arginase was also observed in J774A.1 macrophages [172]. In this study it was additionally shown that the magnitude of this shift was inversely related to the extracellular L-arginine concentration. Similarly, Tenu *et al*. [173] showed on murine peritoneal macrophages that the availability of L-arginine determines whether substrate competition between arginase and iNOS is of functional significance.

Polyamines, mediators derived from the arginase product L-ornithine have also been shown to inhibit NO synthesis in activated J774.2 [174-176] and rat alveolar macrophages [177], In both cell types spermine was much more effective than spermidine, and putrescine had no or only a marginal effect. Spermine suppressed the expression of iNOS, but in addition also that of CAT-2B and this correlated with inhibitory effects on L-arginine transport [177]. A number of cellular effects of polyamines are mediated by aldehyde metabolites rather than by the intact polyamines and evidence was obtained that the inhibitory effects on iNOS and CAT-2B, i.e. on NO synthesis and L-arginine transport, are also mediated by the aldehyde of spermine [175-177]. Finally, it was shown that inhibition of ODC enhanced LPS induced iNOS induction and NO synthesis in J774 macrophages indicating that polyamines formed in the macrophages themselves may play a role in the control of their NO synthesis [178].

On the other hand L-NOHA, the intermediate product in NO synthesis, is a potent inhibitor of arginase I and II (Table **2**) and there is evidence that during iNOS-mediated NO synthesis sufficient amounts of L-NOHA may "escape", resulting in cellular concentrations producing significant inhibition of arginase [76,171,179]. The counter regulatory nature of the NOS pathway on one side and the arginase / polyamine pathway on the other side is additionally underlined by the observations that NO is an effective inhibitor of ODC as demonstrated in various epithelial cells [180-184]. There is evidence that this effect is mediated via nitrosylation of the enzyme [183,184].

7. GPRC6A, A G-PROTEIN-COUPLED RECEPTOR AS TARGET FOR CATIONIC AMINO ACIDS AND REGULATORS OF NITRIC OXIDE SYNTHASE AND ARGINASES

GPRC6A is a subtype of the novel family C of G-protein-coupled receptor (GPCR) with so far unknown physiological function [185-187]. GPRC6A has been characterized as a promiscuous L-α-amino acid receptor with preference for basic amino acids [188]. GPRC6A appears to couple to Gq and activation of the receptor triggers increase in cellular calcium levels. As summarized in Table 2, L-arginine appears to be a slightly more potent agonist than L-ornithine. Most interestingly however, the endogenous arginase inhibitor L-NOHA with an EC_{50} of 10 μM was even more potent than L-arginine (EC_{50} of 24 μM, Table 2). Since in culture medium of activated rat macrophages concentrations between 7-40 μM of L-NOHA were detected [76] it appears likely that in the vicinity of cells in which iNOS has been induced extracellular L-NOHA may reach levels high enough to activate GPRC6A receptors. Moreover, the arginase inhibitor L-nor-NOHA and with somewhat lower potency also the NOS inhibitors (L-NMMA=L-NOARG>L-NAME) showed to be agonists at GPRC6A receptors. This agonism should have implications in the interpretation of experiments in which these tools were used to describe the significance of NOSs and arginases. Interfering effects at GPRC6A have to be considered, particularly as the physiological function of the GPRC6A receptor is not yet understood [189].

Table 2. Agonist Potency of Cationic Amino Acids and Inhibitors of Arginase and NOS at the GPRC6A Receptor

	EC_{50} (μM)
L-Arginine	24
L-Ornithine	45
L-NOHA	10
L-nor-NOHA	19
L-NMMA	55
L-NOARG	54
L-NAME	344

L-NAME, L-N^G-Nitroarginine methyl ester; L-NOARG , L-N^G-Nitroarginine; L-NMMA, L-N^G-Mono-methylarginine; L-NOHA, N^{ω}-hydroxy-L-arginine; L-Nor-NOHA. Data from Christiansen *et al*. [189]

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