Review

CellPress

Arginase: an old enzyme with new tricks

Ruth B. Caldwell[1,2,3], Haroldo A. Toque[4], S. Priya Narayanan[2,3,5], and R. William Caldwell[2,4]

[1] Veterans Affairs Medical Center, One Freedom Way, Augusta, GA 30904, USA
[2] Vision Discovery Institute, Medical College of Georgia, Georgia Regents University, 1459 Laney Walker Boulevard, Augusta, GA 30912, USA
[3] Vascular Biology Center, Medical College of Georgia, Georgia Regents University, 1459 Laney Walker Boulevard, Augusta, GA 30912, USA
[4] Department of Pharmacology and Toxicology, Medical College of Georgia, Georgia Regents University, 1459 Laney Walker Boulevard, Augusta, GA 30912, USA
[5] Department of Occupational Therapy, School of Allied Health Sciences, Medical College of Georgia, Georgia Regents University, 1459 Laney Walker Boulevard, Augusta, GA 30912, USA

Arginase has roots in early life-forms. It converts L-arginine to urea and ornithine. The former provides protection against NH_3; the latter serves to stimulate cell growth and other physiological functions. Excessive arginase activity in mammals has been associated with cardiovascular and nervous system dysfunction and disease. Two relevant aspects of this elevated activity may be involved in these disease states. First, excessive arginase activity reduces the supply of L-arginine needed by nitric oxide (NO) synthase to produce NO. Second, excessive production of ornithine leads to vascular structural problems and neural toxicity. Recent research has identified inflammatory agents and reactive oxygen species (ROS) as drivers of this pathologic elevation of arginase activity and expression. We review the involvement of arginase in cardiovascular and nervous system dysfunction, and discuss potential therapeutic interventions targeting excess arginase.

Arginase, a ubiquitous enzyme

Arginase is a manganese metalloenzyme that catalyzes the conversion of L-arginine to L-ornithine and urea. It is found in bacteria, yeasts, plants, invertebrates, and vertebrates, and is thought to have appeared first in bacteria [1]. The subsequent transfer of arginase to a eukaryotic cell has been suggested to have occurred through mitochondria. Most invertebrates, plants, bacteria, and yeasts have only one form of arginase that is localized in mitochondria. Vertebrates and other animals that metabolize excess nitrogen as urea express a second cytosolic isoform. The cytosolic and mitochondrial arginase isoenzymes are named A1 and A2, respectively. The mitochondrial A2 isoform is thought to be derived from the ancestral arginase because A1 is restricted to a subset of more recently evolved species.

Human A1 consists of 322 amino acids [2] and A2 has 354 amino acids [3]. The two isoforms are encoded by distinct genes on separate chromosomes, but share more than 50% of their amino acid residues, with 100% homology in areas crucial for enzymatic function [4]. High-resolution crystallography has shown that both consist of three identical subunits with an active site at the bottom of a 15 Å deep cleft. Manganese ions, essential for enzyme activity, are located at the bottom of the cleft. The overall fold of each subunit belongs to the α/β family and consists of a parallel, eight-stranded β-sheet flanked on both sides by numerous α-helices [5].

The two arginase isoforms have similar mechanisms, requirement of manganese as a cofactor, and identical metabolites. A1 is cytoplasmic and mainly expressed in the liver. A2 is mainly located within mitochondria and highly expressed in kidney. Arginases can be expressed in many different cell types and can be induced by a wide variety of agents and conditions, depending on tissue and species. Both isoforms are found in the endothelium of the vasculature. Arginase activity has two major homeostatic purposes: first, to rid the body of ammonia through urea synthesis, and, second, to produce ornithine, the precursor for polyamines and prolines (Box 1, Figure 1) [6]. Polyamines produced through ornithine decarboxylase (ODC) are necessary for cell proliferation and the regulation of several ion channels. Proline produced through ornithine aminotransferase (OAT) is necessary for the production of collagen [7,8]. Although there is functional redundancy of the arginase isozymes, inherited defects in A1 can lead to severe and even lethal health problems.

L-arginine is a semi-essential amino acid because it is normally provided through protein turnover, but in some cases it is required from the diet. Acute administration of supplemental L-arginine is reported to prevent or reverse endothelial dysfunction and restore endothelium-dependent vasodilation in diabetes, hypertension, and heart failure. However, several studies in animals and humans

Corresponding authors: Caldwell, R.B. (rcaldwel@gru.edu); Caldwell, R.W. (wcaldwel@gru.edu).
Keywords: arginase; vascular dysfunction; neurodegeneration; polyamine; oxidative stress; nitric oxide; peroxynitrite; superoxide.

0165-6147/
 http://dx.doi.org/10.1016/j.tips.2015.03.006

Box 1. Arginase–ornithine pathway in health and disease

In the liver, A1 catalyzes the last step in the urea cycle, whereby the body disposes of ammonia produced by protein catabolism (see Figure 1 in main text). Ornithine is converted to L-citrulline by the activity of ornithine transcarbamylase (OTC) and carbamoyl phosphate synthase-1 (CPS-1), and to polyamines (putrescine, spermidine, and spermine) and proline by ornithine decarboxylase (ODC) and ornithine amino transferase (OAT), respectively. Polyamines play an important role in cell growth and proliferation, and are involved in wound healing, tissue repair, and neural development [101,102]. Proline is required for collagen formation [103]. In most cell types L-citrulline is recycled back to L-arginine by argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL). ASS and ASL are a portion of the urea cycle [104]. However, most non-hepatic tissues lack CPS-1 or OTC, and therefore do not have the complete urea cycle. Dysregulation of urea cycle enzymes can result in hyperammonemia which, if left untreated, will cause seizures, mental disorders, and early morbidity [105]. Treatment of patients with A1 deficiency involves decreasing protein intake, dietary supplementation with essential amino acids, and in severe cases orthotropic liver transplant [105]. The adverse effects of A2 mutations are not well studied, but A2 knockout mice have been found to develop hypertension [106], and the presence of a rare 2 allele has been linked to an increase in the risk of AD in men and with an earlier age at onset for both genders [72].

The acute phase of wound repair involves oxidative insult through the activation of resident macrophages which express high levels of NOX2 NADPH oxidase and inducible nitric oxide synthase (iNOS), and produce cytotoxic levels of superoxide and NO. Superoxide and NO are important for eradicating pathogens [101]. The acute phase is followed within 3–5 days by a repair phase in which arginase is upregulated [107]. As explained above, arginase converts L-arginine to L-ornithine which is metabolized by OAT, to form proline needed for collagen synthesis, and by ODC to form polyamines which enhance cell proliferation. The balance between rate of consumption of L-arginine by iNOS (for NO production) and arginase (for synthesis of collagen and polyamines) determines the course of wound repair.

Polyamine production by arginase is also important for neural growth, development, and regeneration [102,108,109]. Elevated arginase activity can promote axon regeneration. Following spinal cord transection, treatment of nerve grafts with acidic fibroblast growth factor has been reported to improve locomotor function by a mechanism involving increases in A1 and spermine within motor neurons and macrophages [110]. However, excessive function of the arginase–ornithine pathway can be damaging in other contexts. For example, arginase-dependent increases in the formation of polyamines and proline can lead to thickened, fibrotic, and stiff blood vessels and airways, hypertrophied and fibrotic hearts and kidneys, and growth of cancers [9,10,111–113]. These effects of excessive arginase activity are pathologically significant in diabetes, hypertension, and aging, and may also play a role in tumor growth.

Many research studies have concentrated on the role of arginase in altering NO production and levels because both arginase and NOS utilize L-arginine as their common substrate. Overactive arginase could lead to a deficiency in L-arginine available to NOS. This could cause uncoupling of NOS, decreased NO production, and increased production of the oxidants, superoxide and peroxynitrite (Box 2 and Figures 2 and 3 in main text), resulting in vascular dysfunction. Accordingly, many studies have correlated vascular endothelial dysfunction with increased levels of arginase activity and expression [12,24,49,114–116]. Increased levels of ornithine resulting from enhanced arginase activity can lead to vascular hypertrophy, fibrosis, and stiffness – important aspects of vascular disease [112]. Arginase also plays important roles in reducing NO production by iNOS [10].

Polyamine metabolism has also been shown to be involved in the pathogenesis of ischemic neuronal injury [117–119]. Amino aldehydes, acrolein, and hydrogen peroxide, which are generated as byproducts during the oxidation of spermine and spermidine by polyamine oxidases (see Figure 4 in main text), are toxic and have been implicated in brain and retinal injury [120]. Arginase activity and expression are increased by inflammatory processes and ROS production associated with disease states [8,47,121].

have found no benefit or worsening of adverse outcomes with prolonged administration of supplemental L-arginine [9]. These negative outcomes may be related to the ability of L-arginine to induce expression/activity of arginase and reduce plasma L-arginine levels.

Enhanced arginase activity and the resultant decreases in L-arginine levels can also impair T cell mediated immune function and allow tumor growth by limiting the supply of L-arginine needed for the formation of cytotoxic levels of NO by iNOS [10]. Increased arginase expression/activity may also limit iNOS expression through reducing L-arginine required for iNOS translation [11].

L-arginine is also the substrate for nitric oxide synthase (NOS) (Box 2, Figure 2). When arginase activity is excessive, it can compete with NOS for their common substrate, L-arginine. When the supply of L-arginine required for NO production is insufficient, NOS will become uncoupled [12,13] and will produce less NO and use more molecular oxygen to form superoxide. The superoxide will react rapidly with any available NO to form peroxynitrite, further decreasing NO and further uncoupling NOS by oxidizing the cofactor BH_4 [14] (Figure 3).

We outline here the role played by arginase in health and disease, with particular emphasis on its involvement in disease and injury conditions that affect the peripheral cardiovascular system and the central nervous system (CNS). Upregulation of arginase expression and activity has been demonstrated in many diseases characterized by cardiovascular dysfunction, but has only recently been recognized as a potential mediator of neurovascular disease and injury in the CNS. The sections that follow summarize recent research on the role of arginase in cardiovascular and neurovascular disease. New pharmacological tools are emerging to modulate the activities or expression levels of arginase, and these will be discussed in the last section.

Arginase in cardiovascular disease

In the time since NO was named 'The Molecule of the Year' in *Science* [15], many cardiovascular disease states have been linked to impaired vascular endothelial cell production of NO. In addition, reduced availability of L-arginine has been implicated in vascular dysfunctions. Realization that enhanced arginase activity might compete with NOS for L-arginine and reduce NO levels fueled several studies on its involvement in states of vascular endothelial dysfunction. Elevated levels of L-ornithine, the product of arginase, also have been shown to be a key factor in vascular smooth muscle hyperplasia, fibrosis, and stiffening. We review below some of the recent evidence for the involvement of these arginase pathways in cardiovascular disease and injury conditions.

Hypertension

Hypertension is a major risk factor in cardiovascular disease. It involves reduced NO levels, increased superoxide production, diminished levels of the eNOS substrate L-arginine and cofactor BH4, and increased expression