Arginase and vascular aging

Lakshmi Santhanam, David W. Christianson, Daniel Nyhan, and Dan E. Berkowitz

Departments of Anesthesiology and Critical Care Medicine and Biomedical Engineering; and Johns Hopkins University
School of Medicine, Baltimore, Maryland; and Roy and Diana Vagalos Research Laboratories, Department of Chemistry,
University of Pennsylvania, Philadelphia, Pennsylvania

Submitted 12 May 2008; accepted in final form 14 August 2008

Santhanam L, Christianson DW, Nyhan D, Berkowitz DE. Arginase and vascular aging. J Appl Physiol 105: 1632–1642, 2008. First published August 21, 2008; doi:10.1152/japplphysiol.90627.2008.—Vascular and associated ventricular stiffness is one of the hallmarks of the aging cardiovascular system. Both an increase in reactive oxygen species production and a decrease in nitric oxide (NO) bioavailability contribute to the endothelial dysfunction that underlies this vascular stiffness, independent of other age-related vascular pathologies such as atherosclerosis. The activation/upregulation of arginase appears to be an important contributor to age-related endothelial dysfunction by a mechanism that involves substrate (L-arginine) limitation for NO synthase (NOS) 3 and therefore NO synthesis. Not only does this lead to impaired NO production but also it contributes to the enhanced production of reactive oxygen species by NOS. Although arginase abundance is increased in vascular aging models, it appears that posttranslational modification by S-nitrosylation of the enzyme enhances its activity as well. The S-nitrosylation is mediated by the induction of NOS2 in the endothelium. Furthermore, arginase activation contributes to aging-related vascular changes by mechanisms that are not directly related to changes in NO signaling, including polyamine-dependent vascular smooth muscle proliferation and collagen synthesis. Taken together, arginase may represent an as yet elusive target for the modification of age-related vascular and ventricular stiffness contributing to cardiovascular morbidity and mortality.

nitric oxide synthase 3; S-nitrosylation; vascular stiffness; L-arginine pools; nitric oxide synthase uncoupling

The central aorta dilates with age (52), while the arterial wall thickness increases even in the absence of atherosclerotic disease (64) mainly as a result of intimal thickening (86). Pathologically, aging is associated with elastin depletion and fragmentation, and collagen deposition (68). These changes result in increased vascular stiffness (48) and central pressure augmentation (67), and they manifest themselves clinically as increased systolic and pulse pressures (35, 91). These vascular changes are important contributors to cardiac changes and vascular-ventricular coupling, and the dysregulation of vital organ blood flow that occurs with aging. Several studies across different populations have clearly demonstrated that increased vascular stiffness is an independent risk factor and predictor of cardiovascular morbidity and mortality (27, 37, 56, 79, 92).

At a cellular and molecular level, the mechanisms that initiate and propagate changes in the aging vasculature are currently the focus of increasing investigation. The histological changes that accrue in the vasculature over many years and decades are generally fixed and irreversible, and they are well recognized as significant contributors to increased cardiovascular morbidity and mortality. However, vascular properties, including vascular stiffness, are also modulated acutely, and the dynamic component of vascular stiffness particularly influences cardiovascular performance. Acutely, the endothelium...
also modulates vessel tone (dynamic tone). Endothelial function is now increasingly recognized as extending beyond that of modulating vasomotor tone, for example, via nitric oxide (NO) production (1). In addition and importantly, the endothelium is now recognized as an important modulator of chronic changes that accrue in the underlying vascular smooth muscle and extracellular matrix. Interestingly, despite the multitude of potential aging-related targets in general and in the vasculature in particular, for the most part, they all converge toward two signaling molecules, NO and reactive oxygen species (ROS) such as the superoxide anion (O$_{2}^•$), which combines with NO to yield peroxynitrite and related biological oxidants. Here, we review nitroso-redox balance and imbalance in age-related endothelial dysfunction, and we examine the role that the manganese metalloenzyme arginase plays in modulating this balance.

**NO IN THE AGING ENDOTHELIUM**

The first seminal observation pointing to a role for arginase in age-related endothelial dysfunction accompanied the discovery that despite an increase in endothelial NO synthase 3 (NOS3) expression in old rat aorta, NO production and downstream signaling (cGMP) was decreased (14). Although a multitude of mechanisms might underlie this somewhat counterintuitive observation, the discovery that arginase (Arg) I is expressed not only in the liver (where it is a rate limiting enzyme in the urea cycle), but also in endothelial cells (99), suggested that this enzyme played a role in the impairment of NO signaling. It was reasoned that arginase, which shares L-arginine as a substrate with NO synthase, might compete for limited substrate and thereby regulate the activity of NOS3 in vascular endothelium. This idea was supported by studies of inflammatory cells (macrophages) (63), gastrointestinal smooth muscle tissue (6), and penile smooth muscle tissue (19) in which arginase inhibition enhanced NO production in and this way enhanced immune and erectile function. To understand how this process might occur in the endothelium, it is critical to understand not only the enzymology of both NOS3 and arginase but also to understand the compartmentalization of these enzymes within the endothelial cells in which they reside. Furthermore, it is critical to understand how intracellular pools of L-arginine are regulated and trafficked so that the mechanisms underlying arginase-NOS3 substrate competition become more apparent.

The NOS3 enzyme exists as a homodimer, and its activity is dependent on Ca$^{2+}$-dependent calmodulin binding, requires protoporphyrin IX, tetrahydrobiopterin (BH$_{4}$), FMN, and FAD as enzyme-bound cofactors, and a Zn$^{2+}$ ion tetrahedrally ligated by two cysteine residues from each monomer. NOS is regulated at a transcriptional, posttranscriptional, translational, and posttranslational level (98). With regard to vascular aging, it is well established that there is a decrease in total NO bioavailability in aged vessels. Age-associated increase in the production of endothelial ROS produced by NADPH oxidase (28), xanthine oxidase (66), and mitochondrial enzymes (20, 83, 84), react with NO to form reactive nitrogen species, thereby quenching/diminishing bioavailable NO. Reduced NOS3 phosphorylation and activation in response to shear stress is another hallmark of the aging vascular phenotype (76). Interestingly, sirtuin (SIRT)-1 dependent deacetylation and activation of NOS3 might establish a direct causal connection between calorie restriction (leads to SIRT-1 activation) and NO production (57). Furthermore, cofactor depletion (particularly BH$_{4}$) might lead to NOS3 uncoupling, which results in production of O$_{2}^•$ by NOS rather than NO (23). Finally, and from the perspective of the review, limitation of L-arginine substrate by arginase activation/upregulation leads to age-dependent impairment of NO production and might lead to NOS3 uncoupling. The phenomenon of L-arginine depletion or arginase activation leading to ROS generation has been described for NOS1 (93) and NOS2 (94) and recently in the context of diabetes-induced vascular disease (69) and atherogenesis for NOS3 (70).

**RECIPROCAL REGULATION OF NOS BY ARGINASE**

Arginase converts L-arginine to L-ornithine and urea, and it is found in mammals as two distinct isoforms, Arg I and Arg II, both of which are widely expressed in many tissues, including the cardiovascular system. In extrahepatic tissues, arginase is thought to be involved in the biosynthesis of L-proline (which is a biosynthetic precursor of collagen) and glutamate, as well as polyamines such as spermine and spermidine; additionally, arginase is involved in the inflammatory and the immune response by competing with NOS for the common substrate, L-arginine. This would have important consequences in the cardiovascular system, where vascular tone and function depend on NO derived from NOS3 activity. In addition to the common substrate, NOS and arginase are functionally related in another manner: N$_{2}^•$-hydroxyarginine, produced as an intermediate in the NOS reaction, is a modest inhibitor of arginase (30).

Direct comparison of the $K_{m}$ values could suggest that the high $K_{m}$ value of arginase (1–20 mM) compared with the low $K_{m}$ value of NOS (1–5 $\mu$M) should not allow for effective competition between these enzymes for their common L-arginine substrate. However, because these two enzymes are competing for a common substrate for catalysis, rather than two protein receptors competing for the binding of a common ligand, a better comparison of arginase and NOS should also include the rate of catalysis for each enzyme. Arginase has a $V_{\text{max}}$ of 1,400 $\mu$mol·min$^{-1}$·mg$^{-1}$, while NOS has a $V_{\text{max}}$ of 900 $\mu$mol·min$^{-1}$·mg$^{-1}$. In such a situation, a direct comparison of the $K_{m}$ values is not an appropriate measure of relative rates of reactions of two enzymes particularly when L-arginine concentration is much higher than $K_{m}$ of NOS (33). Assuming Michaelis-Menten kinetics and NOS activity independent of all other cofactors, it is possible to calculate the relative L-arginine consumption rates for NOS and arginase at different molar ratios of NOS/arginase at and different concentrations of L-arginine (Fig. 1). The following features of the NOS-arginase reciprocal regulation become clear: 1) NOS activity is higher at high NOS/arginase molar ratios and low L-arginine concentrations; 2) the two enzymes have comparable activities at lower NOS/arginase molar ratios; 3) the two enzymes have comparable activities at higher L-arginine concentrations, irrespective of the NOS/arginase molar ratio; and 4) arginase activity exceeds NOS activity at higher L-arginine concentrations at all NOS/arginase molar ratios and at all L-arginine concentrations at low NOS/arginase molar ratios. Indeed, it is evident that arginase is capable of competing for the L-arginine substrate. Furthermore, at any L-arginine concentration, arginase inhibi-
tion is, in essence, a shift toward higher NOS/arginase molar ratio, and hence it is a shift toward higher NOS/arginase relative activity.

The concept that L-arginine pools are compartmentalized is emerging, although incompletely understood, helps in the understanding of the reciprocal regulation of NOS by arginase. A complete characterization of this phenomenon has been hindered by the ability to measure local cellular concentrations of L-arginine. However, data from Ellen Closs’s group (17, 74) have demonstrated that endothelial cells contain three pools of L-arginine: The first, pool I, is regulated by the cationic transporter and can be depleted by the cationic amino acid L-lysine. In contrast, pool II is accessible to endothelial NOS but is not freely exchangeable with extracellular L-lysine (or L-arginine). In endothelial cells, there are two components of pool II: IIA and IIB. Pool IIA can be depleted by neutral amino acids and results from recycling of citrulline to arginine, with argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL) being critical enzymes in this process. Interestingly, given that this pool is an important source of L-arginine for NOS, recent data demonstrate that NOS3 is colocalized with ASS and ASL, suggesting that arginine-recycling enzymes, as one would predict, are close to and coupled with the enzyme that utilize the recycled L-arginine (75). How is it that the neutral amino acid exchanger can deplete a component of pool II (IIA)? It is proposed that the neutral amino acids, glutamine, histidine, and asparagine, transstimulate the exchanger with a resultant efflux of citrulline and a subsequent decrease in citrulline recycling (74). On the other hand, pool IIB results from protein breakdown and is not responsive to either cationic or neutral amino acids. Arginase, specifically Arg II in mitochondria, utilizes this pool. This arginase pool IIB is unaffected by extracellular L-arginine. However, this pool of L-arginine might be influenced by arginase and thus modulate the local concentrations of L-arginine available to NOS3. Furthermore, Topal et al. (82) recently demonstrated that depletion of freely exchangeable L-arginine pools using extracellular L-lysine did not modulate the influence of arginase on endothelial cell NO release. This suggests the presence of different L-arginine pools, at least one of which is accessible to NOS and arginase but is not exchangeable with extracellular L-arginine. Thus both enzymology and local substrate concentrations might explain the reciprocal regulation of NOS by arginase through substrate limitation. For a summary schematic outlining the different L-arginine pools, see Fig. 4.

ARGINASE STRUCTURE, FUNCTION, AND INHIBITOR DESIGN

The X-ray crystal structure of rat Arg I provided the first view of the binuclear manganese cluster required for catalysis (47), and the subsequently determined structures of human Arg I (26) and human Arg II (12) revealed essentially identical metal clusters and active site architectures. In each arginase isozyme, the binuclear manganese cluster activates a bridging hydroxide ion for nucleophilic attack at the L-arginine guanidinium group to yield a tetrahedral intermediate, which then collapses to yield L-ornithine and urea (Fig. 2A).

Structural studies of complexed arginase with boronic acid analogs of L-arginine provide important mechanistic inferences on the catalytic mechanism (16). Both (2S)-amino-6-hexanoic acid (ABH) and S-(2-boroethyl)-L-cysteine (BEC) contain trigonal planar boronic acid moieties in place of the trigonal planar guanidinium group of L-arginine (Fig. 2B) (5, 49). On binding to the active site of arginase, the electron-deficient boron atoms of ABH and BEC undergo nucleophilic attack by the metal-bridging hydroxide ion to yield a tetrahedral boronate anion, just as a nucleophilic attack of hydroxide ions at the guanidinium group of L-arginine yields a tetrahedral intermediate. This binding mode is confirmed in X-ray crystallographic structure determinations of enzyme-inhibitor complexes (12, 19, 49); to illustrate, the structure of the human Arg I-ABH complex (K_d = 5 nM) is shown in Fig. 2C, and a schematic showing enzyme-inhibitor interactions is presented in Fig. 2D.

Notably, other amino acids bearing side chains that mimic the tetrahedral intermediate in the arginase mechanism, such as...
an aldehyde that binds as a tetrahedral gem-diol (73), or a
tetrahedral sulfonamide that binds as the ionized sulfonamidate
anion (13), bind 50,000 times more weakly than amino acids
bearing boronic acid side chains such as ABH or BEC. Thus
the chemical properties of the boronic acid side chains of ABH
and BEC are optimally matched to the chemical reactivity of
the metal-bridging hydroxide ion in the arginase active site.

Interestingly, another class of arginase inhibitors consists of
amino acids bearing \( N \)-hydroxyguanidinium side chains, such as \( N \)-hydroxy-L-arginine (NOHA; \( K_i = 10 \mu M \)) (11). Although
NOHA is a modest inhibitor of arginase, its shorter analog
\( N \)-hydroxy-nor-L-arginine (nor-NOHA) is more potent, with
\( K_i = 500 \) nM (80). The crystal structures of the complexes

between rat Arg I and NOHA or nor-NOHA reveal that the
\( N \)-hydroxy group of each inhibitor displaces the metal-bridging
hydroxide ion of the native enzyme (18). Although the \( N \)-hydroxycyanidine amino acids exhibit slightly weaker affinities in
binding to arginase compared with the boronic amino acids, both
classes of inhibitors serve as useful tools for probing the chemical
biology of arginase and arginase/NOS competition for the
L-arginine substrate in various disease states.

**IMPLICATIONS FOR ARGINASE/NOS REGULATION**

Age-related vascular changes have been investigated in
humans and in a number of animal species. In general, it is

---

**Fig. 2.**

A: arginase catalyzes the nucleophilic attack of a metal-bridging hydroxide ion at the guanidinium group of L-arginine to yield L-ornithine and urea. B: boronic acid analogues of L-arginine, 2(S)-amino-6-hexanoic acid (ABH) (\( X = \text{CH}_2 \)) and 5-(2-boronoethyl)-L-cysteine (BEC) (\( X = \text{S} \)), undergo nucleophilic attack by the metal-bridging hydroxide ion in the arginase active site to yield tight-binding boronate anions. C: electron density map of ABH bound to human arginase I, confirming the binding of the tetrahedral boronate anion. [From Di Costanzo et al. (26).] D: scheme showing enzyme-inhibitor hydrogen bond (black dashed lines) and metal coordination interactions (green dashed lines) observed in arginase I-ABH complexes. [From Cox et al. (19) and Di Costanzo et al. (26).] On average, shorter enzyme-inhibitor hydrogen bond interactions are observed in the human arginase I-ABH complex (black numbers) compared with the rat arginase I-ABH complex (orange numbers). This structural feature may account for the higher affinity of ABH toward human arginase I (\( K_d = 5 \) nM) compared with rat arginase I (\( K_d = 110 \) nM).
believed that the relative contributions of dysregulated mechanisms to vascular pathobiology in aging may be species-dependent. Moreover, the contribution of vascular control mechanisms, both in health and in aging, and in disease conditions, is influenced by the specific regional vascular bed and the specific vessel type/size within a vascular bed that is being investigated. While arginase is increasingly recognized as an important mechanisms regulating vasomotor tone, its relative contribution may vary depending on the vascular bed and the vessel size and type. For example, in aging rats, arginase inhibition did not decrease blood flow in muscle resistance arterioles (23). Moreover, BH4 increased brachial artery flow-mediated dilatation in sedentary elderly individuals compared with that observed in young humans, suggesting that arginase may play little or no role (34), although redundancy of mechanisms cannot be ruled out, nor an interaction between BH4 and arginase. A further example of the differential influence of species and vessel type is the observation that BH4 was observed to be decreased in old rat skeletal vessels (23), but it was not increased in the aorta of old mice (10). Notwithstanding, the influence of species, vascular bed and vessel size, and unresolved questions regarding the theoretical underpinnings of the reciprocal regulation of NOS by arginase, there is now sufficient evidence in multiple organ systems and in multiple pathophysiological scenarios to indicate that reciprocal regulation of NOS by arginase is an important mechanism underlying vasomotor regulation, especially in aging and disease states. The process of reciprocal regulation of NOS by arginase has in fact been demonstrated in the majority of cell types/organisms in which NO is an important signaling molecule, including the cardiac myocyte (78), penis (8), airway (58), skin [where it is an important modulator of skin blood flow (40, 41)], inflammatory mediator cells such as macrophages (63) and importantly, the endothelium (7). Furthermore, upregulation of arginase activity has been demonstrated to contribute to the vasoregulatory dysfunction associated with systemic (25, 39, 46, 99) and pulmonary hypertension (43, 61, 96), diabetes and erectile dysfunction (8, 9), impaired bronchodilatory function in asthma (62), and vascular dysfunction in aging (7, 71, 90). These advances have in large measure been a function of the design and synthesis of specific arginase inhibitors such as ABH, BEC, and nor-NOHA.

Our laboratory’s initial observations in an aging rat model suggested that Arg I was the predominant but not exclusive isoform upregulated in rat aorta (7). Furthermore, the cellular source of the upregulated enzyme was confined predominantly but not exclusively to the endothelium. Further studies revealed that upregulated Arg I was indeed confined to the endothelium and that knockdown of Arg I with specific oligonucleotides could restore NO production and endothelium-dependent vasorelaxation in old rat aorta in organ chamber experiments toward that of young rats (90).

The mechanisms underlying increases in arginase expression and/or activity have not been extensively investigated. Studies in rat aortic vascular smooth muscle demonstrated that interleukin-4 and -13 induced upregulation of Arg I was mediated by 10.220.33.5 on June 6, 2017 http://jap.physiology.org/ Downloaded from
ARGINASE AND VASCULAR AGING

Review

by the JAK/STAT6 pathway and associated with increased DNA synthesis and cell proliferation (88). In the porcine coronary arterioles, oxidant stress induced by hydrogen peroxide impairs endothelial-dependent dilation. This effect was associated with increased Arg I DNA and protein expression, and it was associated with increased arginase protein synthesis (81). The specific mechanisms underlying posttranslational modification and increased arginase activity have also not been clearly defined. Given the interaction between NOS and arginase signaling, we hypothesized that S-nitrosylation of Arg I might be an important posttranslational modification mechanism that regulates its activity. The protein sequence for human Arg I contains three cysteines, C45, C168, and C303, with C303 being very close to residue 308 and the COOH terminal S-shaped tail of arginase. The latter mediates 54% of the intersubunit interactions to form the active arginase homotrimer (47, 54, 59). While the monomeric forms of arginase are active, they display only 15–25% activity compared with the trimer (54). We therefore studied S-nitrosylation of each cysteine residue of human Arg I, as well as the effect of S-nitrosylation on enzyme activity and the stability of the Arg I trimer in vitro and ex vivo. Finally, we examined whether altered arginase nitrosylation could contribute to the pathobiology of vascular aging by limiting NO bioavailability, thereby contributing to impaired endothelial function. Aging is well recognized to be associated with an increase in oxidative stress and NOS2 expression (14, 21). Thus we determined whether NOS2 plays a role in arginase upregulation and vascular dysfunction in aging. We have demonstrated that Arg I is activated by nitrosylation of C303; that this activation results from increased stabilization of the arginase trimer; and that nitrosylation is NOS2 dependent. Moreover, this nitrosylation and upregulation of arginase contributes to the endothelial dysfunction in aged vessels (Fig. 3A). Arginase inhibition significantly improves but does not completely reverse age-related endothelial dysfunction. This suggests, not surprisingly, that other mechanisms, both endothelial dependent and independent (e.g., advanced glycation end products) contribute to the aged phenotype. Improved endothelial-dependent relaxation following arginase inhibition is associated with increased NO production (Fig. 3B). NOS2 was not found in younger vessels but was clearly demonstrated in both the endothelial and nonendothelial components of old vessels (Fig. 3C). Increased arginase activity was clearly demonstrated in the endothelial component of old vessels only (Fig. 3D). Importantly, this increased arginase activity was completely reversed by N-[3-aminomethylbenzyl]acetamidine (1400W), a specific NOS2 inhibitor (Fig. 3D).

So what is the connection between arginase activation, NOS2 induction and aging? It is well established that NOS2 is coinduced in response to inflammatory stimuli mediated by a variety of cytokines (51, 60, 63) in rat and mouse models. While there is limited evidence for NOS2 expression in cultured endothelial cells treated with proinflammatory cytokines and lipopolysaccharide, studies have demonstrated the expression of NOS2 in cultured human umbilical venous endothelial cells under various conditions, including vasoactive stimuli (72), infection with Pseudomonas aeruginosa (4), and upreg-

Fig. 4. L-Arginine (L-Arg) pools in the context of the metabolic network of L-arginine in cytosol and mitochondria, including NO synthesis and arginase activity. The concept of specific subcellular L-arginine pools is complex and is dependent not only on the knowledge of the specific membrane cationic transporters but also on an understanding of the networks for L-arginine metabolism in both the cytoplasmic and mitochondrial compartments as well as its coupling to urea synthesis. The picture that seems to be emerging is summarized in the above figure. Pool I is freely exchangeable with extracellular L-arginine and is regulated by the cationic transporter (CAT). This pool can be depleted by exchanging the pool with the cationic amino acid lysine. Pool II, on the other hand, is not exchangeable with extracellular L-arginine and cannot be depleted by extracellular lysine. Part of pool II, pool IIIA, appears to be a function of the recycling of citrulline to arginine with arginosuccinate synthetase (ASS) and arginosuccinate lyase (ASL) being critical enzymes. Ornithine is converted to citrulline by ornithine transcarbamylase (OTC). Finally, the remaining component of pool II, pool IIIB (not shown) cannot be depleted by the neutral amino acids such as histidine (perhaps by transstimulation/exchange and depletion of recycling citrulline) through the system N, neutral amino acid (NAA), transporter (SN1) and is likely provided by protein breakdown. ORNT1, ornithine/citrulline exchanger (transporter); CYT, cytoplasm; MIT, mitochondria.
lation of tissue factor by anti-phospholipid antibodies (85). The expression of NOS2 in brain endothelial cells has been shown during embryonic and postnatal development (36), after ischemic injury (44), and Alzheimer’s disease (29). While a recent study shows expression of NOS2 in endothelial cells overlaying stage 3 human atherosclerotic plaque (97), alterations in the expression of NOS2 in aging human aortic endothelial cells is yet to be examined. Furthermore, a recent study has supported the role that arginase plays in mediating loss of NO and the pathogenesis of endothelial dysfunction in human blood vessels exposed to proinflammatory conditions, specifically inflammatory bowel disease (42). This, in combination with the enhanced expression of proinflammatory markers and cytokines, as well as their receptors, suggests that the contribution of arginase to endothelial dysfunction may be a function of the degree of the vascular inflammatory response to aging. While the rat is a well-accepted model of cardiovascular aging, there is now evidence that upregulation of arginase in humans might contribute to age-related vascular endothelial function (40, 41). This evidence arises from studies in human skin. Reflex cutaneous vasodilation in response to heat is mediated in part by NO signaling, which is significantly attenuated in aging. Interestingly, arginase inhibition in combination with the antioxidant ascorbate or L-arginine significantly restores the reflex responsiveness toward the young phenotype, supporting a role for arginase in endothelial dysfunction associated with aging. Because ascorbate reduces nitrosothiols, it is possible that the effect observed might be a function not only of arginase inhibition but also of a reduction of the NOS2-dependent S-nitrosylation-mediated activation of arginase by nitrosothiol reduction.

The idea that arginase upregulation in aging compromises endothelial function is not limited to the systemic vasculature. Bivalaqua et al. (8) have recently demonstrated that Arg I is upregulated in the corporal tissue of aging mice and rats. Furthermore, both systemic and cavernosal inhibition of arginase, with the specific inhibitor ABH, results in markedly improved erectile function in response to electrical stimulation of the penile nerve. Given these observations regarding restoration of endothelial NO production and endothelial function in multiple beds, it seems likely that arginase represents an important therapeutic target for age-related endothelial dysfunction in which nitroso-redox balance is disturbed. This is relevant to all tissues in which the endothelium plays a critical physiological role, including blood vessels and erectile tissue. While the inhibition of arginase represents a potential risk for suppressing the urea cycle, this remains theoretical because the concentration of enzyme in the liver is 100–1,000 times that present in the endothelium, and it is unlikely to be suppressed by clinically relevant doses of inhibitor. The possibility of inhibition of white blood cell arginase and its effect in immunity also remain theoretical and awaits the results of clinical trials.

SPATIAL CONFINEMENT OF NO SIGNALING, ARGINASE, AND L-ARGININE POOLS

Nitrosylation of Arg I is NOS2 dependent and leads to its activation. The modification promotes L-arginine depletion and reduced NOS3 activity, contributing to endothelial dysfunction associated with aging. However, several key features of this regulatory mechanism remain poorly understood. For example, how does the S-nitrosylation by NOS2 occur in a specific and spatially confined manner? S-nitrosylation of Arg I by NOS2 should cause increased competition with NOS2 for L-arginine, leading to reduced NOS2 activity, and therefore should be a self-limiting step. How then does the balance of nitrosylated Arg I and NOS2 shift in the aging endothelium? Is this caused
by translocation of Arg I away from NOS2? Furthermore, based on the spatial confinement of NO signaling, how does cystosolic Arg I constrain the activity of caveolin-bound NOS3? We proposed that this specificity is determined by NOS2 interacting with Arg I, allowing the nitrosylation to occur. The hypothesis is based on the observations of the Snyder group (50), who have demonstrated that NOS2 binds to and activates cyclooxygenase 2, a proinflammatory protein. Furthermore, another recently published manuscript describes the interaction of NOS2 with phospholipase A2, a critical enzyme in the synthesis of proinflammatory prostaglandins (95). Thus NOS2 seems to activate proinflammatory mediators. In as yet unpublished experiments, we have demonstrated a direct interaction between NOS2 and Arg I based on coimmunoprecipitation experiments. The exact sites of this interaction remain to be determined. So what of the mechanism underlying Arg I-mediated constraint of NOS3 in aging and inflammation? Recent data in red blood cells suggest that Arg I has a binding partner, flotillin (Flot)-1, which promotes translocation of Arg I to the cell membrane (45). Flot-1 is a scaffolding protein and is an integral component of caveolae and lipid rafts (15, 87). Both Flot-1 and Flot-2 are expressed in endothelial cells (15, 77) and could represent a mechanism for the trafficking of Arg I from cytosolic loci to caveolae/lipid rafts in endothelial cells, thus placing Arg I in close proximity to NOS3. The translocation of proteins from one cellular domain to another as a result of S-nitrosylation has been previously demonstrated. For example, S-nitrosylation of GAPDH triggers apoptosis by promoting its binding to Siah1, which translocates to the nucleus (38). In unpublished data, we demonstrated a direct interaction between Arg I and flotillin. We have demonstrated that Arg I interacts with flotillin and in this way might traffic Arg I into a domain in which it constrains NOS3 activity by substrate l-arginine depletion.

**NON-NO-DEPENDENT EFFECTS OF ARGINASE ACTIVATION/ UPREGULATION AND THE POTENTIAL CONSEQUENCES FOR VASCULAR AGING**

While the focus of this review has been on the reciprocal regulation of NOS by arginase as a result of substrate limitation, the effects of arginase activation/upregulation might extend well beyond this effect (Fig. 4). Ornithine, the product of the conversion of l-arginine to urea is the precursor for the synthesis of the primary amino acid, proline, and the polyamine-derived amino acids, spermidine and spermine, that could be important in altering vascular properties (30). Proline, a primary amino acid that is critical in the synthesis of structural proteins and collagen in particular, is produced by the conversion of ornithine by ornithine aminotransferase. Furthermore, the overexpression of Arg I or Arg II in endothelial cells is associated with increased production of proline (55). In addition, the predicted production of collagen in response to stimuli such as tumor growth factor-β and cyclic strain are dependent on proline formation (31, 32). Ornithine decarboxylase converts l-ornithine to the polyamine putrescine with the subsequent synthesis of spermidine and spermine. These polyamines are critical in vascular smooth muscle proliferation, with overexpression enhancing and pharmacological inhibition preventing this proliferative effect (89). Although the effects of arginase on these parameters have not been directly measured in models of aging, it is conceivable that arginase activation might contribute to age-dependent vascular stiffening and pathology through vascular smooth muscle proliferation, as well as collagen deposition.

**IN SUMMARY**

Figure 5 summarizes the potential mechanisms by which arginase might be upregulated and how this may contribute to age related vascular stiffness. The activation of arginase appears to be an important contributor to age-related endothelial dysfunction by a mechanism that involves substrate limitation for NOS3. Not only does this lead to impaired NO production but also it might contribute to the enhanced production of reactive oxygen species by NOS. While arginase abundance might be increased in vascular aging models, it appears that posttranslational modification by S-nitrosylation of the enzyme enhances its activity as well. The S-nitrosylation is mediated by the induction of NOS2 in the endothelium. What remains to be understood involves the mechanism by which NOS2 is induced. Does it involve the activation of receptors for advanced glycation end products as a result of age-dependent accumulation of endothelial advanced glycation end products, or is it dependent on the activations of toll receptors involved in innate immunity and inflammatory responses? Also, arginase activation might contribute to aging-related vascular changes by mechanisms that are not directly related to changes in NO signaling, including polyamine-dependent vascular smooth muscle proliferation and collagen synthesis. Taken together, arginase may represent an as yet elusive target for the modification of age-related vascular and ventricular stiffness contributing to cardiovascular morbidity and mortality.

**GRANTS**

This work is supported in part by National Institutes of Health Grants R01 AG-021523 (to D. E. Berkowitz) and R01 GM-49758 (to D. W. Christianson).

**REFERENCES**


