

# Arginase: Structure, Mechanism, and Physiological Role in Male and Female Sexual Arousal

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## ABSTRACT

Mammalian arginases I and II require an intact binuclear manganese cluster for the hydrolysis of L-arginine to generate L-ornithine and urea. Although arginase isozymes differ in terms of their tissue distribution, cellular localization, and metabolic function, each employs a metal-activated hydroxide mechanism for catalysis. To date, the best arginase inhibitors are those bearing *N*-hydroxyguanidinium or boronic acid “warheads” that can bridge the binuclear manganese cluster. Strikingly, the trigonal planar boronic acids undergo nucleophilic attack by hydroxide ion to form tetrahedral boronate anions that mimic the tetrahedral intermediate and its flanking transition states in the arginase mechanism. Given their affinity and specificity for arginase, boronic acid inhibitors are especially useful for probing the role of arginase in living systems. Arginase can regulate L-arginine bioavailability to nitric oxide synthase by depleting the substrate pool for NO biosynthesis, so arginase inhibition can enhance the substrate pool for NO biosynthesis. Accordingly, arginase inhibition can enhance NO-dependent physiological processes, such as the smooth muscle relaxation required for sexual arousal: administration of arginase inhibitors *in vitro* and *in vivo* enhances erectile function and engorgement in the male and female genitalia. Therefore, arginase is a potential therapeutic target for the treatment of sexual arousal disorders in men and women.

## Introduction

Arginase is a 105 kD homotrimeric enzyme that requires manganese for the hydrolysis of L-arginine to form L-ornithine and urea. Two genetically distinct isozymes have evolved with differing tissue distributions and subcellular locations in mammals.<sup>1,2</sup> Arginase I is found predominantly in the liver, where it catalyzes the final cytosolic step of the urea cycle and is responsible for the generation of ~10 kg of urea per year by the average human adult.<sup>3,4</sup> Arginase II is a mitochondrial enzyme that does not appear to function in the urea cycle and is more widely distributed in numerous tissues, for example, kidney, brain, skeletal muscle, and liver.<sup>5,6</sup> Genetic “knockout” experiments suggest that arginase II functions in L-arginine homeostasis by regulating L-arginine concentrations for cellular biosynthetic reactions such as nitric oxide (NO) biosynthesis.<sup>7</sup> Since L-arginine is the substrate of

both arginase and NO synthase, arginase activity can effectively inhibit NO-dependent processes by depleting the substrate pool available for NO biosynthesis (Figure 1), for example, as observed in the cellular immune response and the regulation of smooth muscle tone.<sup>8–12</sup> Conversely, arginase inhibition can effectively enhance NO biosynthesis and NO-dependent processes by enhancing L-arginine bioavailability to NO synthase.<sup>13–17</sup>

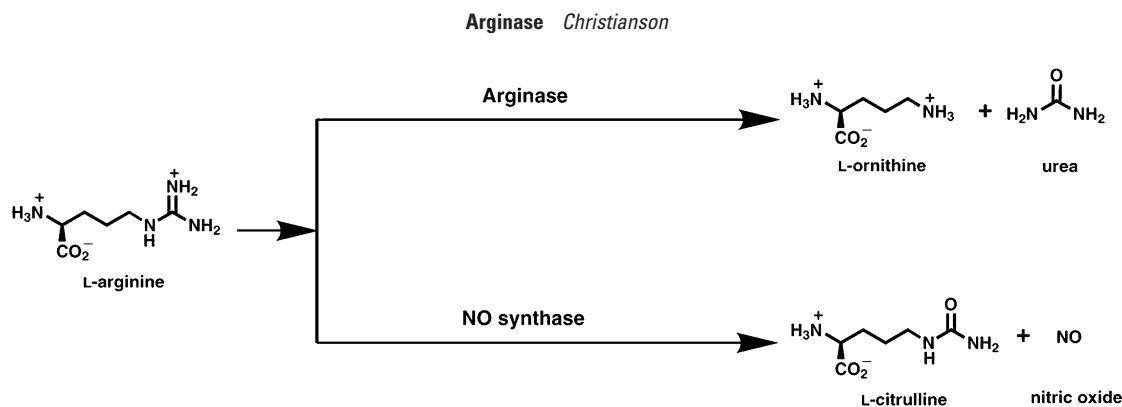
Given that NO-dependent smooth muscle relaxation in the male and female genitalia is required for erectile function and genital engorgements, and given that both NO synthase and arginase are localized in male and female genitalia,<sup>14–16,18–22</sup> it follows that arginase inhibition may enhance the NO-dependent physiological processes required for sexual arousal.<sup>14–16</sup> In this Account, I shall review the structural and chemical biology of arginase, the structure-based design of arginase inhibitors, and the use of these inhibitors to probe arginase function in the physiology of sexual arousal.

## Structure and Chemical Mechanism

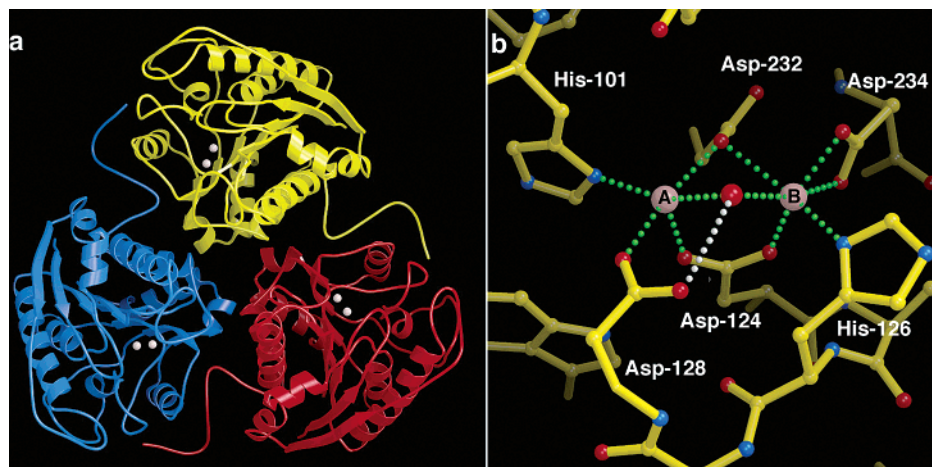
The X-ray crystal structure<sup>23</sup> of rat arginase I provided the first view of the spin-coupled binuclear manganese cluster detected in electron paramagnetic resonance (EPR) experiments (Figure 2).<sup>24</sup> The metal cluster resides at the bottom of a ~15 Å-deep active site cavity in each monomer and the Mn<sub>A</sub><sup>2+</sup>–Mn<sub>B</sub><sup>2+</sup> internuclear separation is 3.3 Å, consistent with the zero-field splitting of the quintet state observed in the EPR spectrum.<sup>25</sup> An intact binuclear manganese cluster is required for the stabilization and orientation of the catalytic nucleophile, a metal-bridging hydroxide ion:<sup>23</sup> the dialysis of Mn<sub>A</sub><sup>2+</sup> from native arginase I or the substitution of ligands to either Mn<sub>A</sub><sup>2+</sup> or Mn<sub>B</sub><sup>2+</sup> in arginase I variants results in up to 20 000-fold reductions in *k*<sub>cat</sub> due to the disruption of the metal cluster.<sup>26–28</sup> Simultaneous coordination of a water molecule to both metal ions in the cluster facilitates ionization to form a metal-bridging hydroxide ion,<sup>29</sup> and the activity-linked p*K*<sub>a</sub> of 7.9 in the pH–rate profile may correspond to this ionization.<sup>30</sup>

Although the variation of Mn<sub>A</sub><sup>2+</sup> or Mn<sub>B</sub><sup>2+</sup> stoichiometry by dialysis or mutagenesis severely compromises catalysis, the Michaelis constant, *K*<sub>M</sub>, remains essentially invariant (1.0–2.5 mM).<sup>26–28</sup> Since *K*<sub>M</sub> reflects enzyme–substrate affinity in the precatalytic enzyme–substrate complex and since *K*<sub>M</sub> is relatively insensitive to perturbations in the binuclear manganese cluster, a strong inner-sphere coordination interaction between the L-arginine guanidinium group and the metal cluster seems unlikely in the precatalytic enzyme–substrate complex.<sup>28</sup> Consistent with this expectation, the recent structure determination of an enzyme–substrate–uncompetitive inhibitor complex confirms a nonmetal binding site for L-arginine (by definition, an uncompetitive inhibitor binds only to the preformed enzyme–substrate complex).<sup>31</sup> Based on analysis of the native enzyme structure, Kanyo and colleagues proposed that the substrate guanidinium group hydrogen bonds

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**FIGURE 1.** L-Arginine catabolism by arginase and NO synthase.



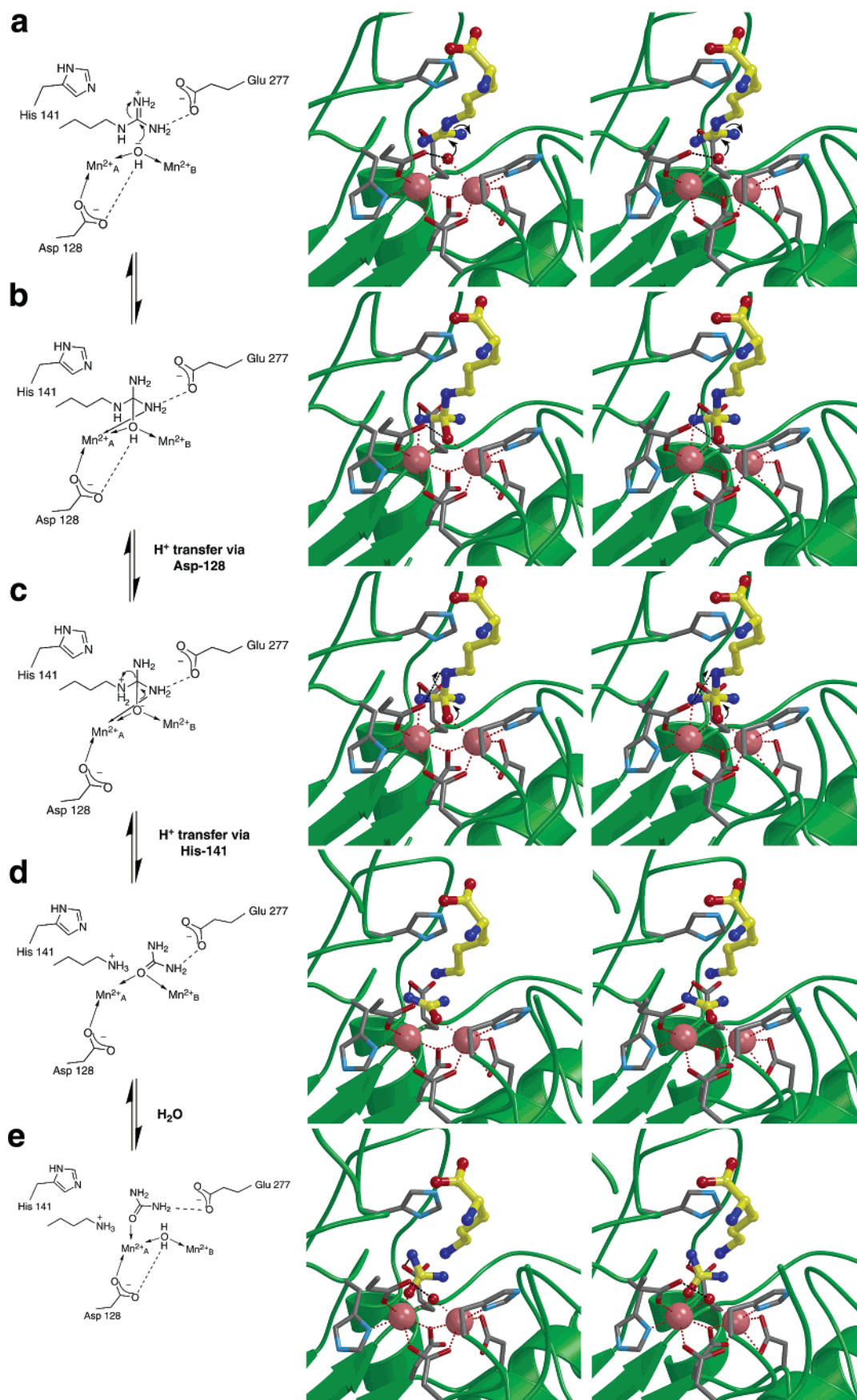
**FIGURE 2.** Panel a presents a ribbon plot of the arginase trimer. The binuclear manganese cluster is represented by a pair of spheres in each monomer. Panel b shows the binuclear manganese cluster of arginase. Metal coordination interactions are indicated by green dotted lines, and the hydrogen bond between the metal-bridging hydroxide ion (red sphere) and Asp128 is indicated by a white dotted line.  $\text{Mn}_A^{2+}$  is coordinated with square pyramidal geometry, leaving a vacant coordination site that permits octahedral coordination geometry as a means of transition state stabilization in catalysis.  $\text{Mn}_B^{2+}$  is coordinated with octahedral geometry. Reprinted with permission from ref 23. Copyright (1996) Nature Publishing Group (<http://www.nature.com/nature/>).

with Glu277.<sup>23</sup> Intermolecular interactions subsequently observed in the crystal structures of rat arginase I, human arginase I, and human arginase II complexed with reactive substrate analogues,<sup>14–16,33</sup> as well as the crystal structure of inactivated *Bacillus caldovelox* arginase complexed with L-arginine,<sup>34</sup> together indicate that the carboxylate side chain of Glu277 and the backbone carbonyl oxygen of His141 accept hydrogen bonds from the guanidinium  $\eta$ 2-NH<sub>2</sub> group, and the hydroxyl side chain of Thr246 accepts a direct or water-mediated hydrogen bond from the guanidinium  $\eta$ 1-NH<sub>2</sub> group.

This hydrogen bond array orients the substrate guanidinium group for nucleophilic attack by the metal-bridging hydroxide ion, which generates a metastable tetrahedral intermediate (Figure 3). Structural studies of arginase complexed with “reaction coordinate analogues” (reactive substrate analogues that undergo a chemical transformation that mimics a chemical step of catalysis<sup>35</sup>) reveal important clues regarding the formation of the tetrahedral intermediate. Consider the boronic acid analogues 2(S)-amino-6-borohexanoic acid (ABH) and S-(2-boronoethyl)-L-cysteine (BEC), in which the trigonal planar boronic acid moiety replaces the trigonal planar

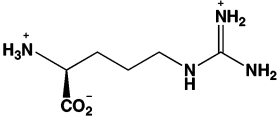
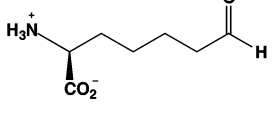
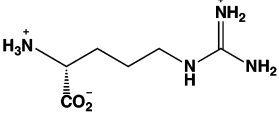
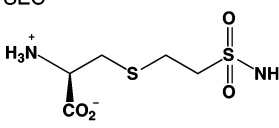
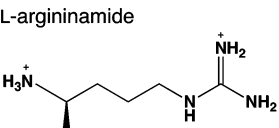
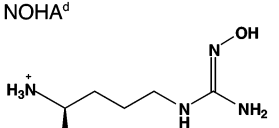
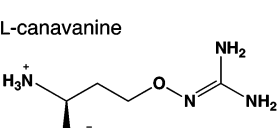
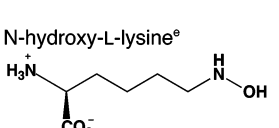
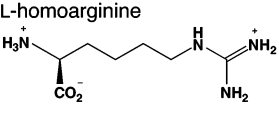
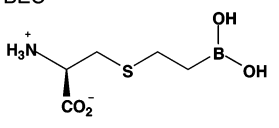
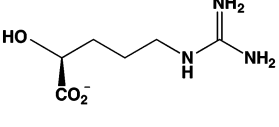
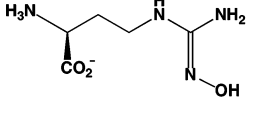
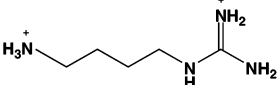
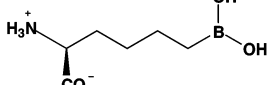
guanidinium group of L-arginine (Table 1).<sup>14,15,36–38</sup> The electron-deficient boron atom in each of these L-arginine analogues is particularly susceptible to attack by a solvent nucleophile, such as the metal-bridging hydroxide ion, to yield the tetrahedral boronate anion—just as nucleophilic attack of hydroxide ion at the guanidinium group of L-arginine yields the tetrahedral intermediate in catalysis.

The crystal structure of rat arginase I complexed with ABH was the first to reveal the molecular strategy for transition state stabilization in catalysis.<sup>14</sup> The tetrahedral boronate anion of ABH (or, by analogy, the tetrahedral intermediate and its flanking transition states) makes multiple interactions with the binuclear manganese cluster and neighboring protein residues (Figure 4): the boronate hydroxyl group O1 symmetrically bridges  $\text{Mn}_A^{2+}$  and  $\text{Mn}_B^{2+}$  ( $\text{Mn}_A^{2+}\cdots\text{O}$  and  $\text{Mn}_B^{2+}\cdots\text{O}$  separations = 2.2 Å) and donates a hydrogen bond to O $\delta$ 2 of D128; the boronate hydroxyl group O2 makes a 2.4 Å coordination interaction with  $\text{Mn}_A^{2+}$  and donates hydrogen bonds to Glu277 and the backbone carbonyl of His141; the boronate hydroxyl group O3 makes a water-mediated hydrogen bond interaction with Thr246. The structures of rat and



**FIGURE 3.** Arginase mechanism. Reprinted with permission from ref 41. Copyright (2001) American Chemical Society.

Table 1. Selected Substrates and Inhibitors of Rat Arginase I

Substrate <sup>a</sup>	$K_M$ (mM)	$k_{cat}/K_M \times 10^3$ ( $M^{-1}s^{-1}$ )	Inhibitor	$K_i$ or $K_d$ ( $\mu M$ )
L-arginine 	1.0	2600	AOH <sup>b</sup> 	60
D-arginine 	—	—	SEC <sup>c</sup> 	52
L-argininamide 	12.4	150	NOHA <sup>d</sup> 	10
L-canavanine 	12.0	7.4	N-hydroxy-L-lysine <sup>e</sup> 	4
L-homoarginine 	7.2	2.8	BEC <sup>f</sup> 	2.2
L-argininic acid 	9.3	0.82	nor-NOHA <sup>d</sup> 	0.5
agmatine 	10.8	0.048	ABH <sup>g</sup> 	0.11

<sup>a</sup> Reference 42. <sup>b</sup> Reference 32. <sup>c</sup> Reference 40. <sup>d</sup> Reference 47. <sup>e</sup> Reference 46. <sup>f</sup> References 15 and 33;  $K_d = 0.27 \mu M$  against human arginase I. <sup>g</sup> References 33, 36–38;  $K_d = 5 \text{ nM}$  against human arginase I and  $K_i = 8.5 \text{ nM}$  against human arginase II.

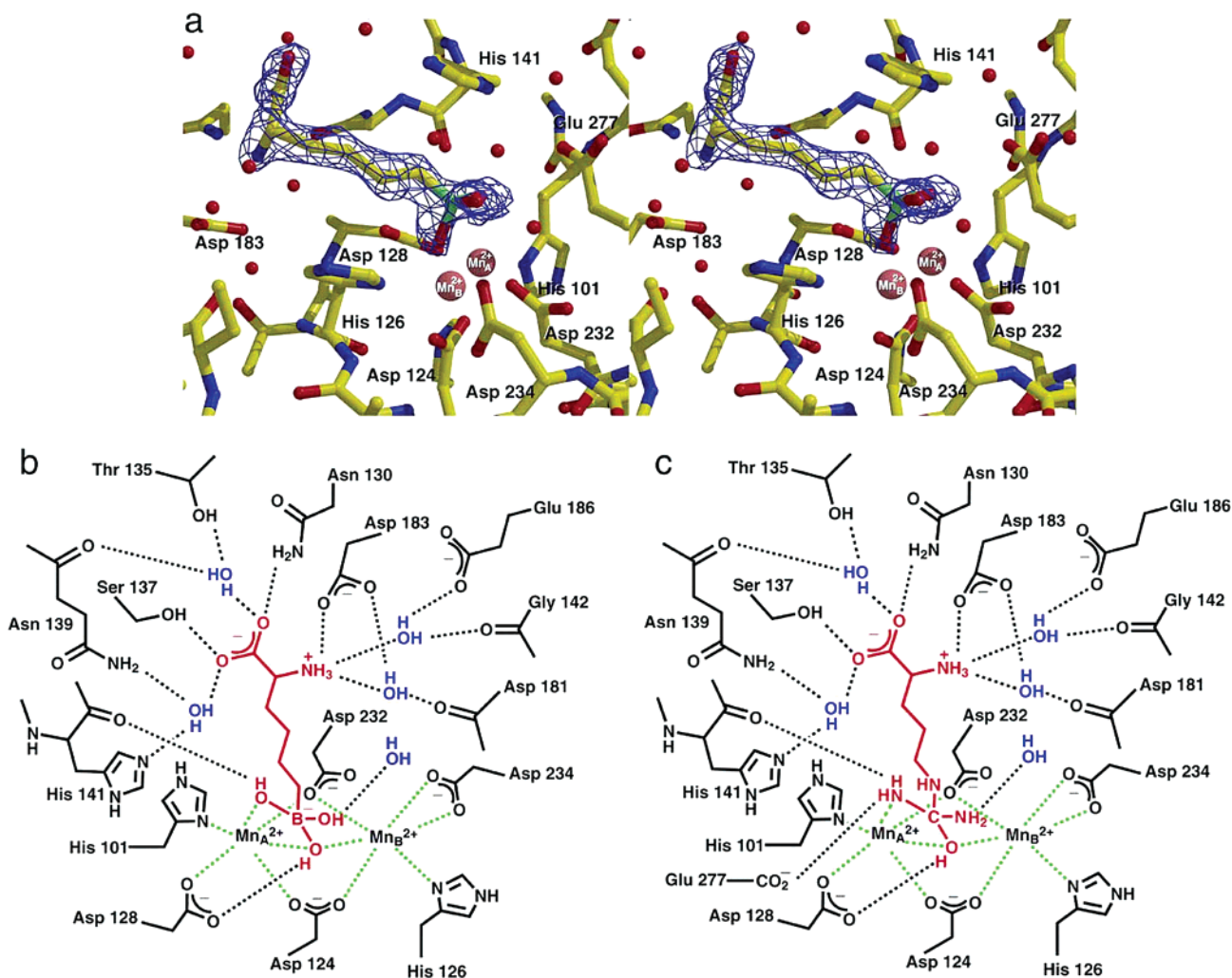
human arginases I and human arginase II complexed with BEC reveal comparable intermolecular interactions.<sup>15,16,33</sup>

Given that ABH binding mimics transition state binding, it is clear that both  $Mn_A^{2+}$  and  $Mn_B^{2+}$  are critical for transition state stabilization: the metal-bridging hydroxyl group of the tetrahedral intermediate (corresponding to boronate hydroxyl group O1) remains close to its position as the former metal-bridging hydroxide ion of the native enzyme, and the vacant coordination site on  $Mn_A^{2+}$  in the native enzyme (Figure 2b) accommodates a sixth coordination interaction by the developing  $sp^3$  lone electron pair on the former  $\eta^2-NH_2$  group of L-arginine (corresponding to boronate hydroxyl group O2) (Figure 3b).

In the crystal structure of the complex between inactivated,  $Mn_B^{2+}$ -depleted *B. caldovelox* arginase and L-

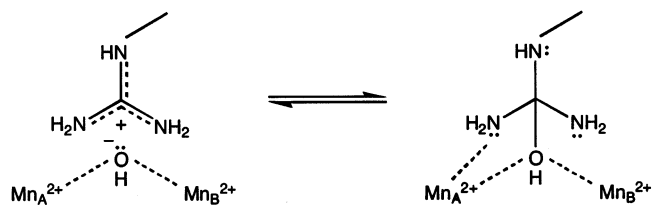
arginine, the substrate  $\eta^2-NH_2 \cdots Mn_A^{2+}$  separation is 2.5 Å.<sup>34</sup> Interpreting this as an inner-sphere interaction, Bewley and colleagues propose that the substrate coordinates to metal in the precatalytic enzyme–substrate complex.<sup>34</sup> However, such an interaction would require the  $\eta^2-NH_2$  group of L-arginine to undergo  $sp^2 \rightarrow sp^3$  rehybridization prior to nucleophilic attack at the guanidinium carbon, which would break the “Y”-shaped guanidinium  $\pi$  system. Moreover, an inner-sphere metal coordination interaction in the precatalytic enzyme–substrate complex is difficult to reconcile with the insensitivity of  $K_M$  to perturbations in the metal cluster.<sup>26–28</sup> Given that the boronate hydroxyl  $O2 \cdots Mn_A^{2+}$  coordination interactions of 2.4 and 2.5 Å in the arginase–ABH and arginase–BEC complexes,<sup>14,15</sup> respectively, are somewhat





**FIGURE 4.** In panel a, the electron density map of the rat arginase I–ABH complex conclusively reveals the binding of ABH as the tetrahedral boronate anion. Panel b shows the intermolecular interactions in the rat arginase I–ABH complex. Metal coordination and hydrogen bond interactions are designated by green and black dashed lines, respectively. Panel c shows postulated intermolecular interactions of the tetrahedral intermediate in arginase catalysis presumed by analogy with panel b. Reprinted with permission from ref 14. Copyright (1999) Nature Publishing Group (<http://www.nature.com/nsmb>).

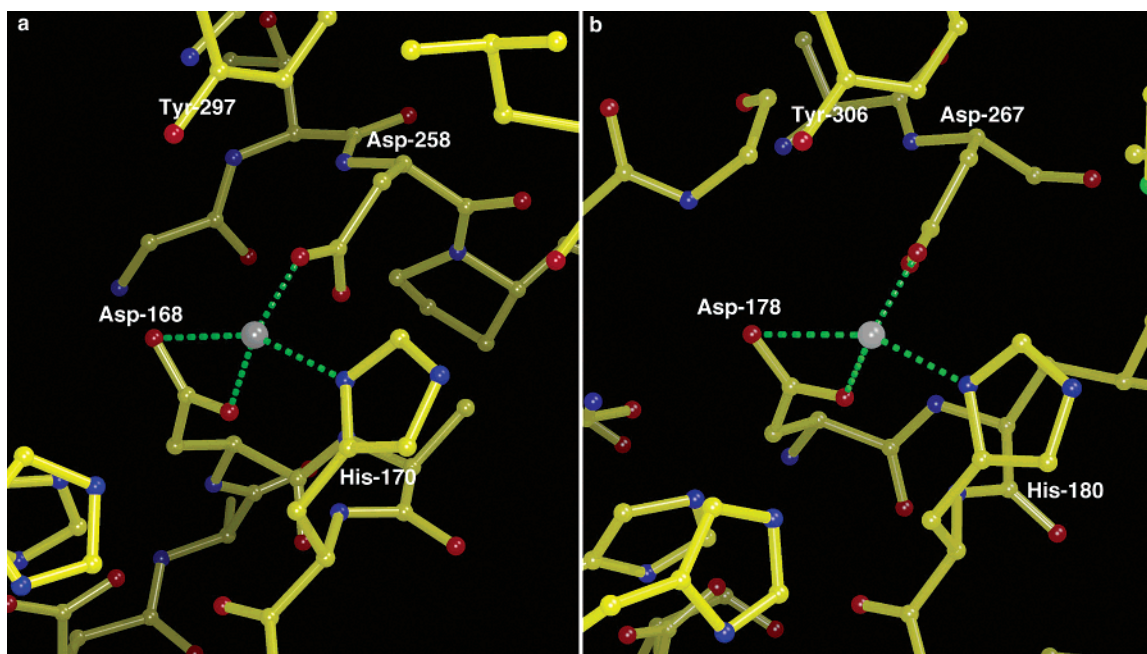
long for inner-sphere interactions and given that the L-arginine  $\eta^2\text{-NH}_2\cdots\text{Mn}_A^{2+}$  interaction of 2.5 Å is comparable,<sup>34</sup> it could be argued that the enzyme–substrate complex involves a weak outer-sphere interaction with  $\text{Mn}_A^{2+}$  if the  $\eta^2\text{-NH}_2$  group of L-arginine undergoes some degree of  $\text{sp}^2 \rightarrow \text{sp}^3$  rehybridization in the active enzyme–substrate complex. Regardless, the  $\text{sp}^3$  lone electron pair on the  $\eta^2\text{-NH}_2$  group is nearly fully developed in the transition state and is fully developed in the tetrahedral intermediate:



That  $\text{Mn}_A^{2+}$  serves a role unique in transition state stabilization illustrates Pauling's notion of transition state stabilization in enzyme catalysis<sup>39</sup> and clarifies the re-

quirement for two manganese ions in arginase catalysis: both metals activate the bridging hydroxide ion nucleophile (Figure 3a),  $\text{Mn}_A^{2+}$  stabilizes the developing  $\text{sp}^3$  lone electron pair on the  $\eta^2\text{-NH}_2$  group as the tetrahedral intermediate is approached (Figure 3b), and both metals stabilize the hydroxyl group of the tetrahedral intermediate and facilitate its ionization prior to the collapse of the tetrahedral intermediate (Figure 3b,c).

Following a proton transfer to the leaving amino group mediated by Asp128, which would be consistent with the binding conformations of boronic acid,<sup>14–16</sup> aldehyde,<sup>32</sup> and sulfonamide<sup>40</sup> analogues, the collapse of the tetrahedral intermediate yields products L-ornithine and urea (Figure 3d). The subsequent addition of a water molecule to the binuclear manganese cluster facilitates urea departure and the complex with L-ornithine, urea, and a metal-bridging water molecule (Figure 3e) has been stabilized for crystallographic analysis.<sup>41</sup> Urea departure may trigger the ionization of the metal-bridging water molecule to regenerate the nucleophilic metal-bridging hydroxide ion. The side chain of His141, which is 4.2 Å



**FIGURE 5.** The zinc binding sites of *A. aeolicus* histone deacetylase-like protein (a) and human histone deacetylase-8 (b) correspond to the  $\text{Mn}_B^{2+}$  site of arginase (Figure 2b).

away from the metal-bridging hydroxide ion in the native enzyme, may help shuttle the ionized proton to bulk solvent.<sup>23</sup>

### Substrate and Inhibitor Specificity

Arginase exhibits exquisite specificity for the hydrolysis of its sole biological substrate, L-arginine. Modifications to substrate structure or stereochemistry significantly attenuate catalysis: D-arginine is not a substrate, and derivatization or deletion of the  $\alpha$ -substituents of L-arginine yields alternative substrates with severely compromised kinetic properties (Table 1).<sup>42</sup> The crystal structures of the rat arginase I–ABH complex<sup>14</sup> and the inactivated *B. caldovelox* arginase–L-arginine complex<sup>34</sup> reveal the structural basis for substrate and inhibitor specificity: an array of direct and water-mediated hydrogen bonds saturate all four acceptor positions on the  $\alpha$ -carboxylate group and all three donor positions on the  $\alpha$ -amino group. These interactions, as illustrated for the rat arginase I–ABH complex in Figure 4b, are generally conserved in the binding of numerous amino acid inhibitors, thereby rationalizing the binding and weak inhibition observed for inert amino acids.<sup>31,43</sup> Interestingly, the majority of hydrogen bond interactions with the  $\alpha$ -substituents are water-mediated, as is the case for the binding of BEC to human arginases I and II.<sup>16,33</sup>

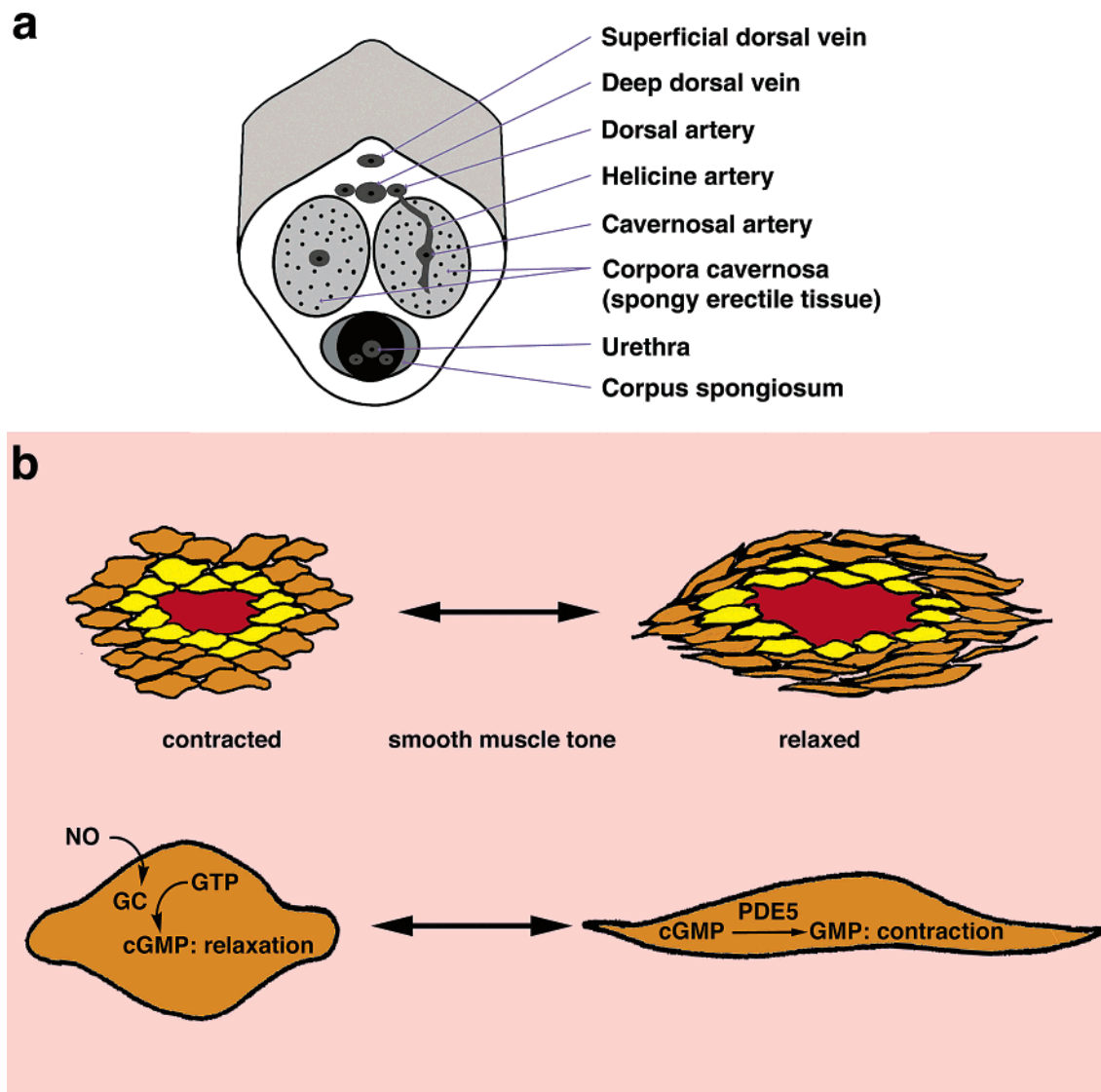
To date, the best arginase inhibitors are those bearing *N*-hydroxyguanidinium or boronic acid “warheads” that bridge the binuclear manganese cluster (Table 1). Intriguingly, an intermediate of NO biosynthesis, *N*<sup>ω</sup>-hydroxy-L-arginine (NOHA; Table 1), is a modest inhibitor of arginase,<sup>44,45</sup> and the crystal structure of its complex with rat arginase I reveals that the *N*<sup>ω</sup>-hydroxyl group displaces the metal-bridging hydroxide ion and bridges the bi-

nuclear manganese cluster.<sup>41</sup> Mansuy and colleagues noted that the distance between the  $\alpha$ -carbon and the *N*<sup>ω</sup>-OH group in a series of *N*<sup>ω</sup>-hydroxy amino acids is critical for inhibitory activity,<sup>46</sup> which led to the design of *N*<sup>ω</sup>-hydroxy-nor-L-arginine (nor-NOHA; Table 1).<sup>47</sup> The crystal structure of the complex between rat arginase I and nor-NOHA reveals that the *N*<sup>ω</sup>-hydroxyl group of the inhibitor displaces the metal-bridging hydroxide ion.<sup>41</sup>

The simplest arginase inhibitor is the fluoride ion,<sup>48,49</sup> which is an uncompetitive inhibitor with  $K_i = 1.3$  mM.<sup>48</sup> By definition, an uncompetitive inhibitor binds to the enzyme–substrate complex, and the crystal structure of the rat arginase I–L-arginine–(F<sup>−</sup>)<sub>2</sub> complex has been determined.<sup>31</sup> This unusual mode of inhibition involves the displacement of the metal-bridging hydroxide ion by a fluoride ion, and the addition of a fluoride ion to the formerly vacant coordination site on  $\text{Mn}_A^{2+}$ . Metal-bound fluoride ions are further stabilized by short hydrogen bond interactions with the substrate guanidinium group, so substrate binding is clearly required to stabilize inhibitor binding: this is the hallmark of an uncompetitive inhibitor.

### Divergence of Arginase and Histone Deacetylase from a Primordial Mononuclear Metalloenzyme

Arginase bears a surprising evolutionary resemblance to the mammalian histone deacetylases (HDACs) and their bacterial homologues, but the HDACs exhibit strikingly different metal ion specificity and stoichiometry for catalysis. The HDACs contain a single  $\text{Zn}^{2+}$  ion that is required for the hydrolysis of acetylated lysine residues located near the N-termini of nucleosomal histones, a process that is generally accompanied by decreased



**FIGURE 6.** In panel a, a cross-section of the human penis illustrates the anatomy of the corpus cavernosum, which becomes engorged with blood during erection. In panel b, smooth muscle relaxation in genital tissues such as the male or female corpus cavernosum results in engorgement and erection. NO activates guanylate cyclase (GC), which generates cyclic guanosine monophosphate (cGMP), which mediates intracellular processes that lead to relaxation. Phosphodiesterase V (PDE5) degrades cGMP and leads to smooth muscle contraction. Inhibitors of PDE5 sustain cGMP concentrations and thereby enhance smooth muscle relaxation (e.g., this is the therapeutic strategy of Viagra). Insufficient NO flux in this signaling pathway could result in insufficient cGMP concentrations, in which case a phosphodiesterase V inhibitor will be unable to enhance smooth muscle relaxation in the absence of enhanced NO flux.

transcriptional activity.<sup>50,51</sup> The catalytic core of class I and class II HDACs is related to the histone deacetylase-like protein (HDLP) from *Aquifex aeolicus* (35% identity), the prokaryotic acetoin utilization proteins (28% identity), and prokaryotic acetylpolymine amidohydrolase (15% identity).<sup>52</sup> The X-ray crystal structure of HDLP unexpectedly revealed the “arginase fold” despite only ~15% amino acid sequence identity, and the recently determined structures of human HDAC-8 reveal the same topological similarity.<sup>53–55</sup>

The crystal structures of HDLP and HDAC-8 show that the catalytic  $Zn^{2+}$  ion binds to a site corresponding to the higher-affinity  $Mn_B^{2+}$  site of arginase:  $Zn^{2+}$  ligands Asp168/Asp178, His170/His180, and Asp258/Asp267 of HDLP/HDAC-8 correspond to  $Mn_B^{2+}$  ligands Asp124, His126, and Asp234 of arginase (compare Figure 5 with

Figure 2b). In HDLP/HDAC-8, the residues corresponding to  $Mn_A^{2+}$  ligands Asp128 and Asp232 of arginase are not conserved, so a “ $Mn_A^{2+}$  site” has not evolved for metal binding in the histone deacetylases. It is notable that the weaker  $Mn_A^{2+}$  site of arginase ( $K_d = 8 \mu M$ )<sup>26,27</sup> corresponds to the more “mutated” metal site in the histone deacetylases, suggesting that the evolution of the  $Mn_A^{2+}$  site and the divergence of  $Zn^{2+}/Mn^{2+}$  specificity occurred after the evolutionary divergence of arginase and histone deacetylase from a common primordial ancestor.

### Arginase and the Physiology of Sexual Arousal

A recent survey indicates that 31% of men and 43% of women aged 18 to 59 years report varying degrees of sexual dysfunction.<sup>56</sup> Rooted in physiological or psycho-

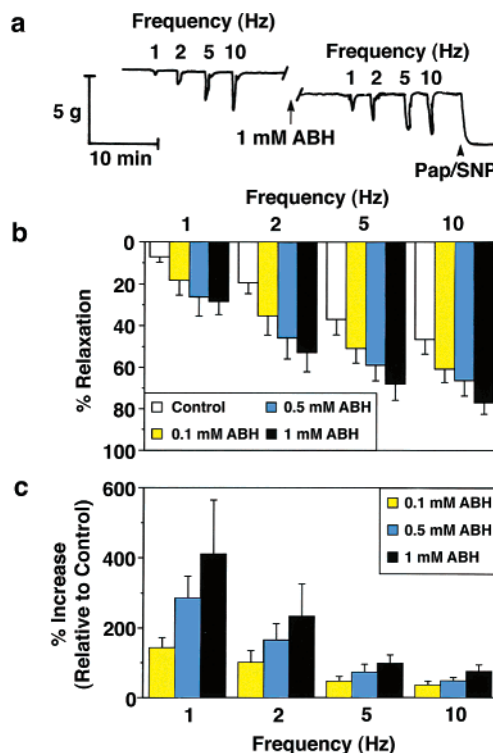


logical causes or both, the various manifestations of this malady present an insidious threat to satisfactory reproductive health. In men, sexual dysfunction is succinctly characterized as erectile dysfunction (impotence), whereas in women, sexual dysfunction is more broadly classified in four main categories: hypoactive sexual desire, sexual arousal disorder, orgasmic disorder, and sexual pain disorder.<sup>57</sup> Female sexual arousal disorder, defined as an inability to achieve or maintain sufficient sexual excitement, including clitoral erection and genital engorgement, is physiologically analogous to male erectile dysfunction in that a deficiency in genital blood circulation compromises the hemodynamics of erection/engorgement.

Erectile dysfunction can result from physiological defects in the complex cascade of enzyme-catalyzed reactions governing blood flow into and out of the corpus cavernosum, a muscularized chamber of expandable tissue that becomes engorged with blood in the erect penis or clitoris (Figure 6a). Nitric oxide is the principal mediator of erectile function<sup>18</sup> and governs nonadrenergic, noncholinergic neurotransmission in penile corpus cavernosum smooth muscle.<sup>19,58–61</sup> NO causes rapid relaxation of smooth muscle tissue and thereby facilitates the engorgement of the corpus cavernosum (Figure 6b). Thus, NO synthase is clearly a critical enzyme in the physiology of sexual arousal.

A growing body of evidence suggests that arginase is also a critical enzyme in the physiology of sexual arousal, since it is coexpressed with NO synthase in smooth muscle tissue.<sup>13–15,62</sup> Given that arginase and NO synthase compete for the same substrate, L-arginine, arginase appears to attenuate NO synthase activity and NO-dependent smooth muscle relaxation by depleting the substrate pool of L-arginine that would otherwise be available to NO synthase (Figure 1).<sup>13–16</sup> Indeed, the overexpression of arginase II in the corpus cavernosum of diabetic men likely contributes to the erectile dysfunction that often accompanies this disease.<sup>63</sup> Conversely, arginase inhibition by the boronic acid inhibitors ABH or BEC (Table 1) sustains cellular L-arginine concentrations,<sup>13</sup> which in turn enhances NO synthase activity and NO-dependent smooth muscle relaxation in tissue bath experiments with rabbit and human penile corpus cavernosum (Figure 7)<sup>14,15</sup> and penile erection in live rabbits (Figure 8).<sup>16</sup> Thus, arginase is strongly implicated as a regulator of erectile function, and human penile arginase is accordingly a potential target for the treatment of male sexual dysfunction.

While the etiology of female sexual dysfunction is more complex, female sexual arousal disorder resulting from compromised circulation in the genitalia might similarly respond to arginase inhibition. Defined as genital arousal disorder, this malady can result from autonomic nerve damage or multiple sclerosis (notably, arginase I is significantly overexpressed in a murine model of multiple sclerosis<sup>17</sup>). Given the localization of NO synthase in clitoral corpus cavernosum<sup>20</sup> and vagina,<sup>21</sup> the subsequent identification of arginase activity in vaginal tissue extracts<sup>16</sup> suggests that arginase may similarly serve to regulate NO-dependent smooth muscle relaxation and engorgement

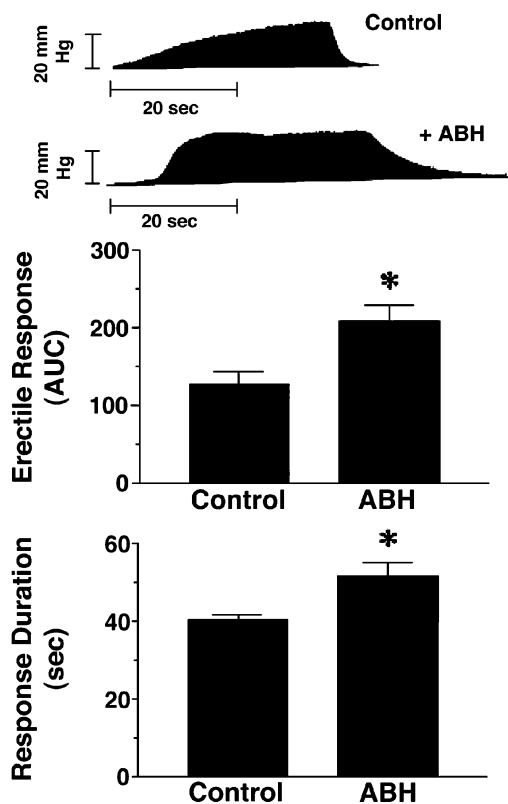


**FIGURE 7.** Effect of ABH on smooth muscle relaxation triggered by electric field stimulation (EFS) in organ bath experiments with penile corpus cavernosum. Panel a shows a representative polygraph tracing of responses to EFS in the absence and presence of 1 mM ABH; tissue tone is represented as grams of tension on the ordinate. In the absence of EFS, ABH causes moderate relaxation due to basal NO synthase activity. Panel b provides a summary of data acquired from four organ bath experiments. Panel c shows total increases in cavernosal smooth muscle relaxation caused by ABH. Reprinted with permission from ref 14. Copyright (1999) Nature Publishing Group (<http://www.nature.com/nsmb>).

in the female genitalia. Administration of the arginase inhibitor ABH to live female rabbits enhances genital engorgement following pelvic nerve stimulation, giving rise to 2-fold increases in genital tissue oxyhemoglobin concentrations as measured by near-infrared spectroscopy<sup>16</sup> and 34% enhancement of vaginal blood flow as measured by laser Doppler flowmetry.<sup>64</sup> Thus, arginase is strongly implicated as a regulator of genital hemodynamics and engorgement in the female, and arginase is therefore a potential target for the treatment of female sexual arousal disorder. Notably, ABH administration has no discernible effect on systemic arterial blood pressure in either male or female rabbits, so its effect on other NO-mediated vascular phenomena appears to be limited.<sup>16</sup>

With L-arginine bioavailability to NO synthase being implicated in male and female sexual arousal, it is perhaps not surprising that dietary supplements (“nutraceuticals”) have been explored as remedies for sexual dysfunction. Dietary L-arginine supplements enhance intracavernosal pressure in the rat,<sup>65</sup> and 6 out of 10 men taking 2800 mg of L-arginine per day subjectively report improved erectile function in a pilot study.<sup>66</sup> In another study, 73.5% of women taking a nutritional supplement containing 2500 mg of L-arginine subjectively report “improved satisfaction





**FIGURE 8.** The arginase inhibitor ABH enhances the erectile response (area-under-the-curve of intracavernosal pressure as a function of time) and the duration of the erectile response following pelvic nerve stimulation in the male rabbit. Reprinted with permission from ref 16. Copyright (2003) American Chemical Society.

with their overall sex life".<sup>67</sup> However, efficacy has generally been limited in well-controlled clinical trials,<sup>68</sup> perhaps due to the "arginase problem"—L-arginine nutraceuticals supply substrate to both arginase and NO synthase, so arginase activity still attenuates NO biosynthesis and NO-dependent physiological effects.

## Concluding Remarks

Although it seems that the advent of Viagra (sildenafil citrate) first popularized frank discussions of sex and chemistry, the molecular mechanisms of sexual arousal have been probed for many years prior. Indeed, Brindley used himself as a test subject for the evaluation of injectable penile erection drugs nearly two decades ago.<sup>69</sup> Since Brindley's pioneering contributions, pharmacological approaches using injectable and topically applied vasodilators such as nitroglycerin or prostaglandin E1 have shown efficacy in the treatment of male and female sexual arousal disorders.<sup>70,71</sup> However, such approaches bypass the neural circuitry between behavior and biochemistry that turns on the NO-dependent physiological processes required for sexual arousal.

Inhibitors of phosphodiesterase V (Figure 6b) are notable in that they do not bypass the neural circuitry of cavernosal smooth muscle relaxation and sexual arousal; instead, phosphodiesterase V inhibitors prevent the degradation of the second messenger, cGMP. Inhibitors of

arginase are likewise notable in that they do not bypass the neural circuitry of sexual arousal; instead, they prevent L-arginine degradation, which then enhances L-arginine concentrations for biosynthesis of the first messenger, NO. Targeting arginase with inhibitors to enhance NO biosynthesis may be a useful strategy for the treatment of other diseases linked to L-arginine homeostasis, such as multiple sclerosis or other diseases of the immune response. For example, in a murine model for human multiple sclerosis characterized by significant arginase I overexpression, ABH-treated mice developed milder disease symptoms with delayed onset, reduced disease score, and expedited recovery.<sup>17</sup> Future research will undoubtedly illuminate additional disease pathologies in which this ubiquitous manganese metalloenzyme is implicated.

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