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Arginase II Deletion Increases Corpora Cavernosa Relaxation in Diabetic Mice

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Abstract

Introduction—Diabetes-induced erectile dysfunction involves elevated arginase (Arg) activity and expression. Because nitric oxide (NO) synthase and Arg share and compete for their substrate L-arginine, NO production is likely linked to regulation of Arg. Arg is highly expressed and implicated in erectile dysfunction.

Aim—It was hypothesized that Arg-II isoform deletion enhances relaxation function of corpora cavernosal (CC) smooth muscle in a streptozotocin (STZ) diabetic model.

Methods—Eight weeks after STZ-induced diabetes, vascular functional studies, Arg activity assay, and protein expression levels of Arg and constitutive NOS (using western blots) were assessed in CC tissues from non-diabetic wild type (WT), diabetic (D) WT (WT+D), Arg-II knockout (KO) and Arg-II KO+D mice (N=8–10 per group).

Main Outcome Measures—Inhibition or lack of arginase results in facilitation of CC relaxation in diabetic CC.

Results—Strips of CC from Arg-II KO mice exhibited an enhanced maximum endothelium-dependent relaxation (from 70±3% to 84±4%) and increased nitrenergic relaxation (by 55%, 71%, 42%, 42%, and 24% for 1, 2, 4, 8 and 16 Hz, respectively) compared to WT mice. WT+D mice showed a significant reduction of endothelium-dependent maximum relaxation (44±8%), but this impairment of relaxation was significantly prevented in Arg-II KO+D mice (69±4%). Sympathetic-mediated and alpha-adrenergic agent-induced contractile responses also were increased in CC strips from D compared to non-D controls. Contractile responses were significantly lower in Arg-II KO control and D versus the WT groups. WT+D mice increased Arg activity (1.5-fold) and Arg-II protein expression and decreased total and phospho-eNOS at Ser-1177, and nNOS levels. These alterations were not seen in Arg-II KO mice. Additionally, the Arg inhibitor BEC (50 μM) enhanced nitrenergic and endothelium-dependent relaxation in CC of WT+D mice.

Conclusion—These studies show for the first time that Arg-II deletion improves CC relaxation in type 1 diabetes.

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Conflict of Interest: None

Introduction

Penile erection is the result of cavernosal smooth muscle relaxation and nitric oxide (NO) is the main neurotransmitter/vasorelaxant agent involved in this process [1]. NO is derived from L-arginine by NO synthase (NOS), and both endothelial NOS (eNOS) and neuronal NOS (nNOS) isoforms serve as sources to produce relevant levels of NO in the corpus cavernosum (CC). Reduced NO bioavailability has been implicated in erectile dysfunction (ED) in many conditions including obesity [2], hypercholesterolemia [3], aging [4] and diabetes [5].

According to the National Health and Nutrition Examination Survey, the rate of ED in diabetic men is much higher than the non-diabetic [6]. Clinical and epidemiological studies have revealed an association between ED and diabetes. This association is characterized by autonomic neuropathy and microvascular system changes that favor vascular dysfunction in diabetic patients [7]. However, the etiology of ED in diabetic patients has several aspects. For diabetic human, rabbit, mice and rat penes, these include impairment in the NO/cGMP signaling pathway and smooth muscle relaxation, which involves reduction of NO production, and the enhancement of vasoconstrictor tone [8,9,10]. Previous studies in our lab and others indicate that diabetes elevates vascular Arg activity, which contributes to endothelial dysfunction [11,12,13]. The role of Arg in modulating NO production is not limited to regular peripheral vascular endothelium. Previous study has shown that human diabetic CC with ED exhibits higher Arg activity, and diminished NO synthesis with reduced cavernosal relaxation [5,14,15]. Furthermore, increased Arg and decreased NOS expression levels are reported in diabetic rats. Additionally, inhibition of Arg has been shown to enhance NO production [16] and improve vasorelaxant responses to acetylcholine in hypertensive, high fat diet and diabetic models [11,17,18], while over expression of Arg-I or Arg-II decreases intracellular L-arginine levels and suppresses NO synthesis [19,20].

Two isoforms of Arg exist, Arg-I and Arg-II. Each is encoded by a separate gene and found in vascular tissues, endothelial and smooth muscle cells, but their distribution appears to be vessel- and species-dependent [21–23]. L-arginine is the substrate required by NOS to produce NO. Arg appears as a critical regulator for NO production by competing with NOS for L-arginine. Related studies have shown that elevated Arg activity/expression in vascular tissues and endothelial cells is linked to cardiovascular diseases. Arg-I has been found to be increased and associated with cell proliferation [24] and endothelial dysfunction in ischemia/reperfusion injury [25], aging [26], and diabetes [11]. In contrast, Arg-II appears to be involved in the pathogenesis of atherosclerosis [18], prostate cancer [27] and ED [5]. Increased expression of Arg-II is evident in diabetic cavernosal tissue, and inhibition of this enzyme facilitates smooth muscle relaxation [5,19].

Earlier studies have established that streptozotocin (STZ) treatment provides a good animal model for type 1 diabetes-induced ED [28]. Since Arg is up-regulated in diabetes, a mechanism for loss of erectile function may be a combination of decreased production and/or bioavailability of NO via increased expression of Arg. The specific Arg isoform responsible for the impaired relaxation of CC appears to be Arg-II. Currently available Arg inhibitors are not isoform-selective, but a knockout mouse strain lacking Arg-II has been developed [29]. Thus, it was hypothesized that deletion of Arg-II would improve CC smooth muscle relaxation function in type 1 diabetes.

Methods

Induction of Diabetes

Protocols were conducted in concordance with the Medical College of Georgia's Animal Use for Research and Education Committee. A total of 78 ten week-old male C57BL/6J mice were used in this study. Mice were divided into four groups (N= 8–10 per group): non-diabetic wild type (WT), Arg-II knockout (Arg-II KO) mice, diabetic WT (WT+D), and Arg-II KO+D mice. In the D group, mice received intraperitoneal injections of streptozotocin (STZ, 65 mg/kg every other day for up to 3 injections). In the non-diabetic groups, citrate buffer (pH 4.5), the vehicle of STZ, was injected in the same manner as in the diabetic groups. Mice with blood glucose levels >450 mg/dL were considered diabetic. Body weight and glucose levels of each mouse were measured at the time of injections and eight weeks after treatment.

Functional Studies

After eight-weeks of diabetes, animals were anesthetized, and the penes were removed and placed in chilled Krebs solution of the following composition (in mM): NaCl, 118; NaHCO₃, 25; glucose, 5.6; KCl, 4.7; KH₂PO₄, 1.2; MgSO₄ 7H₂O, 1.17 and CaCl₂ 2H₂O, 2.5. After removal of the vein and urethra, the penile tissue was cleaned from connective and adventitial tissue, and the fibrous septum separating the corpora cavernosa was opened from its proximal extremity toward the penile shaft. A slit was made in the tunica albuginea along the shaft to obtain two strips (approximately 11 × 1 × 1 mm) of CC from each animal. Each strip was mounted under resting tension of 2.5 mN in 4-ml myograph chambers filled with Krebs solution at 37°C (pH 7.4) and continuously bubbled with a mixture of 95% O₂ and 5% CO₂. Isometric force was recorded using a Power Lab/8SP data acquisition system (Software Chart, version 5, AD Instrument, Colorado Springs, CO, USA). The tissues were allowed to equilibrate for 1 h before starting the experiments.

After equilibration, the ability of the preparation to develop contraction was assessed by a high KCl solution (80 mM). Cumulative concentration-response curve to acetylcholine (ACh; 10⁻⁹ to 10⁻⁵ M), an endothelium-dependent vasodilator were obtained in cavernosal strips contracted with phenylephrine (PE; 10⁻⁵ M, α₁-adrenergic receptor agonist). Then, cavernosal tissues were washed three times every 15 min for 1 h. Next, strips from WT mice were incubated with BEC (5 × 10⁻⁵ M, arginase inhibitor) for 30 min before a second curve was generated. Cumulative concentration-response curve to sodium nitroprusside (SNP; 10⁻⁸ to 10⁻⁴ M, NO donor) and the contractile agent PE (10⁻⁹ to 10⁻⁴ M) were also performed in the cavernosal tissue.

In another set of experiments, electrical field stimulation (EFS) was applied in cavernosal strips placed between two platinum ring electrodes connected to a grass S88 stimulator (Astro-Med, Industrial Park, RI, USA), and EFS was conducted at 20 V, 1-ms pulse width and trains of stimuli lasting 10s at varying frequencies (1–32 Hz). To evaluate nitrergic relaxations, cavernosal tissues were pretreated with bretylium tosylate (3 × 10⁻⁵ M) and atropine (10⁻⁶ M) to deplete the catecholamine stores and to block the muscarinic receptors, respectively. Involvement of NO on EFS-induced cavernosal relaxations was confirmed by using L-NAME (10⁻⁴ M, NO synthesis inhibitor). To confirm the role of Arg in diabetes, frequency-response curves were performed in the absence or in the presence of acute treatment of BEC (5 × 10⁻⁵ M for 30 min) in cavernosal tissues. To evaluate adrenergic nerve-mediated responses, the strips were incubated with L-NAME (10⁻⁴ M) plus atropine (10⁻⁶ M), before EFS was performed.

Arginase Activity Assay

Mice cavernosal tissues were frozen in liquid nitrogen, pulverized, combined 1:4 (wt:vol) with ice-cold lysis buffer (5×10^{-2} M Tris-HCl, 10^{-4} EDTA and EGTA, pH 7.5) containing protease inhibitors, phosphatase cocktail 1 and 2 and homogenized on ice. The homogenate was sonicated and centrifuged at 14,000 g for 20 minutes at 4°C and the supernatant was removed for enzyme assay. 25 µL of the supernatant was collected in triplicate and then added to 25 µL of Tris-HCl 121 (5×10^{-2} M, pH 7.5) containing 10^{-2} M MnCl₂ and the mixture was activated by heating for 10 minutes at 55–60°C. Arg activity was assayed by measuring urea produced from L-arginine using a colorimetric assay previously described [2].

Western Blot Analysis

Tissues were homogenized in lysis buffer containing protease and phosphatase inhibitors, and centrifuged at 14,000 g for 20 minutes at 4°C, supernatant collected and protein concentration determined. Protein (20 µg) was resolved on a 10% SDS-polyacrylamide pre-cast gel and transferred to polyvinylidene difluoride membrane. The membrane was blocked in advance blocking agent (Amersham) and then incubated with primary antibody (anti-arginase I, BD Transduction Laboratories, 1:1000; anti-arginase II, Santa Cruz Biotechnology, INC, 1:250; anti-eNOS, anti eNOS phosphorylated at Ser¹¹⁷⁷ and at Thr⁴⁹⁵, Cell Signaling Technology, 1:1000; anti nNOS, BD Transduction laboratories, 1:4000) in Tris-buffered saline/Tween 20 buffer overnight at 4°C. p-eNOS examined in this assay was not purified. After washing, the membranes were incubated with sheep anti-mouse (Amersham, 1:4000) or donkey anti-rabbit (GE Healthcare, 1:4000) horseradish peroxidase-labeled secondary antibody, respectively, and visualized using an enhanced chemiluminescence kit (Amersham, Piscataway, NJ, USA). The protein expression levels were normalized by α -actin.

Drugs and Chemicals

Acetylcholine, atropine, bretylium tosylate, N^ω-nitro-L-arginine methyl ester (L-NAME), sodium nitroprusside, streptozotocin, phenylephrine, phosphatase cocktail 1 and 2 and protease inhibitor were purchased from Sigma Aldrich (St Louis, MO, USA). The arginase inhibitor (S)-(2-boronoethyl)-L-cysteine hydrochloride (BEC, HCl) was purchased from Calbiochem (EMD Biosciences, Inc, La Jolla, CA, USA). All reagents used were of analytical grade. Stock solutions were prepared in deionized water or ethanol and stored in aliquots at −20°C; dilutions were prepared immediately before use.

Statistical Analysis

Experimental values of relaxation or contraction were calculated relative to the maximal changes from the contraction produced by PE and KCl, respectively, taken as 100% in each tissue. Data are shown as the mean $\pm$ SEM of the mean of *n* experiments. Statistical comparisons were made using two-way analysis of variance (ANOVA) followed by bonferroni post hoc test. *P* < 0.05 was considered significant. A program package was used for the statistical analysis of all data (GraphPad InStat, version 5.00; GraphPad Software Inc., San Diego, CA).

Results

Plasma Glucose Concentrations and Body Weight

Diabetes caused an approximate four-fold increase in blood glucose levels (*P* < 0.001) and decreased body weights in WT and Arg-II KO mice significantly by 19% and 15%, respectively (Table 1). The average dry weights (milligrams) of the cavernosal strips did not

differ significantly between all groups. Contractile responses to a high K^+ solution (KCl 80 mM) was not different among all groups (WT, 1.39 ± 0.25 mN; Arg-II KO, 1.64 ± 0.15 mN; WT+D, 1.45 ± 0.17 mN; Arg-II KO+D, 1.32 ± 0.22 mN).

Arginase II Deletion Ameliorates Cavernosal Relaxation to Acetylcholine (ACh) and Electrical Field Stimulation (EFS) in Diabetes

Cumulative addition of ACh (10^{-9} to 10^{-5} M, Figure 1A) as well as frequency-dependent nerve stimulation (EFS, 1–32 Hz) produced relaxation of cavernosal strips precontracted with phenylephrine (PE 10^{-5} M) in all groups ($N = 8$ each group). The maximal response (E_{max}) and potency (pEC_{50}) values to ACh for each group are listed in Table 2. Although no differences were observed in the pEC_{50} values to ACh between non-diabetic WT, Arg-II KO or Arg-II KO+D groups, WT+D group displayed lower pEC_{50} (2.1-fold) and E_{max} (reduced by 37 %) values compared to WT group ($P < 0.05$). Arg-II KO mice exhibited greater relaxant responses to EFS (by 55%, 71%, 42%, 42% and 24% at 1, 2, 4, 8 and 16 Hz, respectively) compared to CC from WT mice ($P < 0.05$; Figure 1B). Furthermore, endothelium-dependent and nitrgic relaxation in the strips from Arg-II KO+D mice was significantly greater than observed in WT+D mice ($P < 0.05$), and were more similar to responses in WT mice (Figure 1A and 1B). Incubation with L-NAME (10^{-4} M) fully blocked ACh- and EFS-induced cavernosal relaxations in all groups (data not shown).

Sodium Nitroprusside (SNP)-Induced Cavernosal Relaxations

The NO donor SNP (10^{-8} to 10^{-4} M) caused concentration-dependent cavernosal relaxations which did not differ among the groups. The pEC_{50} and E_{max} values for the SNP are shown in Table 2.

Arginase II Deletion Prevents Diabetes-Induced Augmented Responses to Phenylephrine (PE) and Adrenergic Nerve Stimulation

PE (10^{-9} to 10^{-4} M) caused concentration-dependent contractions in cavernosal preparations in all groups. The pEC_{50} and the E_{max} values elicited by PE were significantly augmented in WT+D compared to WT mice ($P < 0.05$, Table 2). Similarly, contractile responses to PE were significantly decreased in the Arg-II KO and Arg-II KO+D groups in comparison to those in WT groups (Figure 2A).

After incubation with L-NAME (10^{-4} M) and atropine (10^{-6} M), EFS produced frequency-dependent contractions in cavernosal strips (1–32 Hz) that were abolished by pretreatment with either an α -adrenoceptor antagonist prazosin (10^{-6} M) or catecholamine depletor, bretylium tosylate (3×10^{-5} M, data not shown). This confirms that nerve-induced contractile responses are mediated by catecholamine release. As shown in Figure 2B, EFS-induced contractions are enhanced in the cavernosal strips from WT+D mice compared with those from non-diabetic WT and Arg-II KO mice. Arg-II KO+D group exhibited significantly lower contractile responses in CC from those of the D group by 36%, 42% and 35% at 8, 16 and 32 Hz, respectively.

Acute Inhibition of Arginase Enhance Endothelium-Dependent- and Nitrgic Relaxation in Diabetes

Acute treatment with BEC (5×10^{-5} M) significantly enhanced the E_{max} to ACh in cavernosal tissues from WT+D mice (from $44 \pm 5\%$ to $59 \pm 5\%$, $P < 0.05$, Figure 3A), and increased the EFS-induced relaxation at 1, 2, 4, and 8 Hz by 225%, 80%, 60% and 43% respectively, in cavernosal tissues from WT+D mice (Figure 3B). BEC did not alter the relaxation induced by ACh, but significantly enhanced the nitrgic relaxation at lower frequencies (by 138%, 66% and 28% at 1, 2 and 4 Hz, respectively) in the WT group. No

enhancement of cavernosal relaxation was observed in non-diabetic or diabetic in Arg-II KO mice after treatment with BEC (Figure 3C and D).

Arginase II Enzyme Is the Primary Isoform Upregulated in Corpora Cavernosal Tissue in Diabetes

A significant increase of Arg activity was observed in the CC tissue from WT+D (by 1.5-fold) compared with non-diabetic WT mice (Figure 4). In contrast, Arg activity was not altered in Arg-II KO+D mice compared to non-diabetic Arg-II KO mice. Arg activity in non-diabetic Arg-II KO mice was not significantly lower than in non-diabetic WT mice.

As expected, Arg-II corporal protein levels were not expressed in Arg-II KO mice. In contrast, Arg-II corporal expression levels were increased in cavernosal tissue from WT+D mice (1.8-fold) compared with non-diabetic WT mice ($P < 0.01$, Figure 5A). On the other hand, no differences were observed in Arg-I protein levels in the corporal tissue from non-diabetic WT or Arg-II KO mice compared with WT+D or Arg-II KO+D mice (Figure 5B).

Arginase II Deletion Increases Constitutive NOS Expression

Diabetes decreased corporal expression levels of nNOS (Figure 6A), and total eNOS and phospho eNOS (at its regulatory sites Ser-1177) as compared with non-diabetic WT mice ($P < 0.05$). However, the levels of phosphorylated Thr-495, which inactivates eNOS, did not differ in the diabetic compared with the non-diabetic mice. Arg-II KO mice exhibited increased levels of nNOS ($P < 0.05$) and phospho eNOS corporal expression (at Ser-1177, $P < 0.01$) when compared to non-diabetic WT mice (Figure 6B and 6C). Deletion of Arg-II prevented the decrease in both phospho eNOS at Ser-1177 and in nNOS in diabetic mice.

Discussion

This study shows that increased corporal Arg activity and Arg-II expression in the CC of diabetic mice is associated with decreased endothelial-dependent and nitric nerve relaxation responses in CC. This condition favors penile detumescence and dysfunction. This study also demonstrates for the first time that removal of Arg-II by gene deletion enhances corpora cavernosa relaxation and prevents vascular dysfunction in a type 1 diabetic model. These responses were associated with enhanced nitric and endothelial-dependent relaxation, increased nNOS and phospho eNOS (at regulatory site Ser-1177) expression in CC tissue, and decreased contractile responses to an α_1 -adrenergic receptor agonist and adrenergic nerve stimulation.

Corporal smooth muscle relaxation is necessary for normal penile erection. Studies indicate that endothelial- and neuronal derived NO mediates this relaxation [1]. Much evidence indicates that diabetes impairs both endothelial and nitric relaxation in the CC isolated from various species [30], that in turns leads to reduced cavernosal smooth muscle relaxation and to ED [8,31]. The present study also showed decreased relaxant responses to acetylcholine and nitric nerve stimulation in the CC from diabetic mice, confirming these early reports. It has been widely accepted that a reduction of NO bioavailability is a major cause of ED in diabetic patients. Considering that diabetes is a leading cause of ED with undefined mechanisms [32,33], reduction of Arg activity may provide a beneficial therapeutic approach for preventing impaired erectile function.

Arg catalyzes the hydrolysis of L-arginine to form L-ornithine and urea. Both isoforms of Arg compete with NOS for their common substrate, L-arginine, and can reduce NO production and increase superoxide production [34]. Depending on the disease state and tissue, Arg-I or Arg-II or both may be up-regulated and exert a prominent action. Previous reports have indicated that an Arg inhibitor enhances NO-dependent relaxation of penile CC

smooth muscle [19]. In this study, it was found that Arg-II gene deletion prevents impairment of endothelium-dependent and nitrgic relaxation function caused by diabetes in the cavernosal tissue of mice. These results indicate that the decline in erectile response that occurs with diabetes can be attributed to Arg-II since expression of this isoform is up-regulated in the penile vasculature while Arg-I levels are unchanged. These data are further supported by studies in diabetic [5] and angiotensin II treated mice [2] where increased Arg-II seems to play a role in ED. Moreover, these data show increased levels of Arg-II activity in CC of WT mice, supporting the concept that cavernosal NO production may be linked to the regulation of Arg activity and expression (II). This study shows that Arg-II gene deletion prevents diabetic-induced elevation of Arg activity in CC tissue. Since Arg activity in CC of non-diabetic Arg-II KO mice was not significantly lower than WT mice, Arg-I in CC of these mice appears to contribute significantly to basal Arg activity. These findings are in contrast with those of Lim et al. [2007], in which aortic Arg activity was significantly reduced in Arg-II KO with no major contribution from the Arg-I isoform.

Since reduced NOS function has been identified in diabetes-induced impaired CC relaxation, protein levels of NOS isoforms were assessed in corpora cavernosa tissues. The constitutive eNOS and nNOS isoforms are tightly regulated and produce physiologically relevant levels of NO in endothelial cells and autonomic nerve endings of the penis, respectively [36]. Previous studies have reported that nNOS is involved in initiating of erection, while activation of eNOS facilitates the attainment and maintenance of full erection [37]. The current results indicate that Arg-II KO mice express higher protein levels of nNOS and p-eNOS at regulatory site Ser-1177 in cavernosal tissues compared to those of WT mice. In contrast, diabetes suppresses expression of nNOS and p-eNOS (at its regulatory sites Ser-1177). Furthermore, diabetic mice lacking Arg-II had increased p-eNOS (at Ser-1177) compared to WT diabetic mice. These biochemical findings are consistent with results of functional studies, where endothelial and nitrgic relaxation responses were enhanced in the Arg-II KO mice, and were diminished in the diabetic mice. Because Arg competes with NOS for L-arginine, up-regulation of Arg activity/expression induced by diabetes may limit L-arginine availability for NOS. Further *in vivo* data are needed to actually determine whether Arg-II deletion improves erectile function in diabetes.

Alteration in eNOS function via posttranslational modifications or protein-protein interaction is involved in a number of disease states that are associated with ED. Phosphorylation of eNOS is a posttranslational mechanism that regulates its activity and NO availability under physiologic circumstances. Previous studies have pointed to diabetes-related changes in eNOS phosphorylation. Cavernosal and aortic preparations from diabetic mice and carotid plaques from diabetic patients displayed decreased phosphorylation at Ser-1177 [38,39,40]. In contrast to Ser-1177, one study shows that eNOS phosphorylation at Thr-495, a negative regulator of eNOS activity, did not change in the diabetic mice compared with the non diabetic mice [40]. Taken together, these previous studies and results from the present study establish that nNOS and eNOS regulation at Ser-1177 are critically important for CC relaxation. Although eNOS phosphorylation at Ser-1177 was enhanced in CC of Arg-II KO mice, this does not exclude the possibility that phosphorylation of other sites could increase or decrease of NOS activity in diabetic CC. Further studies are needed to determine whether other phosphorylation sites of eNOS are involved in the penis.

Previous studies have shown that inhibition of Arg restores endothelial NO signaling and L-arginine responsiveness in diabetic and aged rats [5,41,42]. Although the present data show that the arginase inhibitor BEC did not alter the maximal response for ACh and EFS in non-diabetic mice, the efficacy of this inhibitor was evident when applied to tissues affected by diabetes. Treatment with BEC partially restored the endothelium-dependent and nitrgic CC relaxation response in diabetic mice when compared to non-diabetic mice. Inhibition of

arginase appeared more effective in enhancing relaxation of CC to nitregic EFS than to ACh. This difference probably relates to the greater physiologic involvement of nitregic function in the erectile process as compared with humoral activation of endothelial NOS.

Although inhibition or lack of Arg improved CC relaxation, a complete restoration of relaxation was not seen in CC from diabetic mice. Increased accumulation of endogenous NOS inhibitors such as monomethylarginine (MMA) and asymmetric dimethylarginine (ADMA) also could be involved in this CC dysfunction. Elevated plasma levels of ADMA have been found in diabetes [43] and associated with ED [44]. Thus, down-regulation of constitutive NOS proteins in concert with increased Arg activity and accumulation of endogenous NOS inhibitors may be involved in diabetes-induced impaired CC relaxation.

Diabetes causes activation of RhoA/Rho kinase (ROCK) pathway in vascular tissue (11, 45). Earlier studies have shown that the active ROCK pathway is involved in increased activity and expression of Arg [11,23,46]. In addition, active ROCK reduces the activity of myosin light chain (MLC) phosphatase in CC vascular smooth muscle, resulting in enhanced contractile tone [15,47]. Thus, lack of full CC relaxation with arginase inhibition also may be due to effects of enhanced RhoA/ROCK activity in smooth muscle MLC.

The mechanism of penile erection involves a delicate balance between relaxing and contractile factors in the corpora cavernosa of the penis. Alteration in this balance has been reported in disease states. Contraction of the cavernosal smooth muscle is maintained by noradrenaline released from noradrenergic nerves and via stimulation of the α_1 -adrenoceptor. This contraction is based on the activation of subcellular signaling system that mobilizes Ca^{2+} from extracellular and intracellular stores, resulting in an increased intracellular Ca^{2+} concentration [48]. Additionally, adrenergic stimulation also activates Ca^{2+} -sensitizing signaling mediated by RhoA/Rho-kinase [47]. The present results showed that PE- and EFS-induced corporal contractions are increased in diabetic mice, and these enhanced contractions are attenuated in Arg-II deficient mice. Augmented sympathetic nerve transmission associated with increased vasoconstrictor action mediated by α_1 -adrenoceptor activation contributes to detumescence and the flaccid state of the penis. Decreased contraction observed in CC from Arg-II KO diabetic mice appears to be related to reduced NO production in the penile vasculature. These findings are in agreement with those of Lim et al., (2007) in aorta, where dampened vasoconstrictor responses by deletion of Arg-II were attributed to decreased NO production. Others have concluded that the decreased contractile responses of aorta from Arg-II KO mice can be attributed to a loss of Rho-kinase activity via lack of Arg-II [49]. A direct action of arginase to regulate ROCK activity has not been reported. However, it is reasonable to project that reduced Arg-II function can down regulate ROCK levels by increasing NOS function and reducing ROS formation.

In conclusion, this study indicates that Arg-II plays an important role in decreasing NO production. Further, the findings highlight the possible beneficial effect of inhibiting Arg-II function in enhancing the CC relaxation and preventing impaired CC relaxation responses in type I diabetes.

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Figure 1. Endothelium-dependent NO-mediated relaxation to acetylcholine (ACh, 10^{-9} to 10^{-5} M, panel **A**) or nitregic nerve stimulation (electric field stimulation, EFS 1–32 Hz, panel **B**) in cavernosal segments from non-diabetic wild type (WT, open circle), diabetic WT (WT+D, open square), arginase II (Arg-II) knockout (KO, closed circle) and Arg-II KO+D (closed square) mice. Data were calculated relative to the maximal changes from the contraction produced by phenylephrine (PE, 10^{-5} M) in each tissue, which was taken as 100%. Data represent the means $\pm$ S.E.M. of 8 experiments. * $P < 0.05$; ** $P < 0.01$, compared with WT mice; # $P < 0.05$; !! $P < 0.01$, compared with WT+D group.

Figure 2. Contractile-response curves upon stimulation of α_1 -adrenergic receptor, phenylephrine (PE, 10^{-9} to 10^{-4} M, panel **A**) or adrenergic nerves (electric field stimulation, EFS 1–32 Hz, panel **B**) in cavernosal segments from non-diabetic wild type (WT, open circle), diabetic WT (WT+D, open square), arginase II (Arg-II) knockout (KO, closed circle) and Arg-II KO +D (closed square) mice. Data were calculated relative to the maximal changes from the contraction produced by KCl (80 mM), which was taken as 100%, and data represent the mean $\pm$ S.E.M. of 8–9 experiments. * P < 0.05; ** P < 0.01, compared with WT mice; # P < 0.05, compared to WT+D mice.

Figure 3.

Effect of the arginase inhibitor BEC (5×10^{-5} M) on the relaxation induced by acetylcholine (ACh, 10^{-9} to 3×10^{-6} M, panel **A** and **C**) or on the EFS-induced relaxations (1–32 Hz, panel **B** and **D**) in the cavernosal strips from non-diabetic and diabetic in wild type (WT) and Arg-II KO mice. Data were calculated relative to the maximal changes from the contraction produced by phenylephrine (PE, 10^{-5} M), which was taken as 100%. Data represent the mean $\pm$ S.E.M. of 5–6 experiments. * $P < 0.05$ and ** $P < 0.01$, compared to WT+D group.

Figure 4.

Arginase (Arg) activity in cavernosal tissues from non-diabetic wild type (WT, open bar), diabetic WT (WT+D, grey bar), Arg-II knockout (KO, hatched bar) and Arg-II KO+D (closed bar) mice was determined in corpora cavernosa by urea production. Data are expressed as % of WT. Data represents the mean $\pm$ S.E.M. of 5 experiments. * $P < 0.05$, compared to WT mice.

Figure 5.

Western blot analysis of arginase II (Arg-II, panel **A**) and Arg-I (panel **B**) in cavernosal tissues of non-diabetic wild type (WT, open bars), diabetic WT (WT+D, grey bars), Arg-II knockout (KO, hatched bars), and Arg-II KO+D (closed bar) mice. A representative blot is shown in the top panel. Results were quantified by densitometry and Arg protein was normalized by α-actin levels expressed as % of WT. Data represents the mean ± S.E.M. of 5 experiments (each group). ** $P < 0.01$, compared to WT mice.

Figure 6.

Western blot analysis of total eNOS, nNOS (panel **A**) and total and phospho eNOS at their regulatory site Ser-1177 (panel **B**) and Thr-495 (panel **C**) in cavernosal tissues of non-diabetic wild type (WT, open bar), diabetic WT (WT+D, grey bar), arginase II (Arg-II) knockout (KO, hatched bars) and Arg-II KO+D (closed bars) mice. A representative blot is shown in the top panel. Protein expression of constitutive NOS were normalized by α -actin levels and expressed as % of WT. Data represents the mean $\pm$ S.E.M. of 5 experiments (each group). * $P < 0.05$; ** $P < 0.01$, compared to WT mice.

Table 1

Body weight, blood glucose and cavernosal strip weight of non-diabetic wild type (WT), diabetic WT (WT +D), arginase (Arg) II knockout (KO) non-diabetic (Arg-II KO) and diabetic (Arg-II KO+D) mice.

	WT	WT+D	Arg-II KO	Arg-II KO+D
<i>Body weight (g)</i>	28.5±2.2	22.9±1.9*	27.8±2.1	23.5±1.9
<i>Blood glucose (mg/dl)</i>	138±32	512±20*	175±36	557±19*
<i>Cavernosal strip weight (mg)</i>	1.89±0.2	1.78±0.3	1.92±0.2	1.83±0.2

* $P < 0.05$, compared with WT mice.

Table 2

Potency (pEC₅₀) and maximal response (E_{max}) values obtained from concentration-response curves to acetylcholine (ACh, 10⁻⁹ to 10⁻⁵ M), sodium nitroprusside (SNP, 10⁻⁸ to 10⁻⁴ M) and phenylephrine (PE, 10⁻⁹ to 10⁻⁴ M) in cavernosal strips from non-diabetic wild type (WT), diabetic WT (+D), arginase (Arg) II knockout (KO) non-diabetic (Arg-II KO) and diabetic (Arg-II KO+D) mice. Data represent the means ± S.E.M. of 8 experiments.

	WT			WT+D			Arg-II KO			Arg-II KO+D		
	pEC ₅₀	E _{max}		pEC ₅₀	E _{max}		pEC ₅₀	E _{max}		pEC ₅₀	E _{max}	
ACh	7.02±0.03	70±3		6.70±0.07*	44±9*		7.08±0.03	84±4*		6.97±0.14	69±4#	
SNP	6.44±0.05	77±5		6.49±0.08	84±6		6.48±0.10	79±7		6.46±0.04	84±6	
PE	5.81±0.03	130±5		5.98±0.08	154±8*		5.36±0.04	112±6		5.61±0.03	128±5	

* P < 0.05 compared with WT mice;
P < 0.05 over compared with D mice.