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 nondiabetic 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 nitrergic relaxation (by 55%, 71%, 42%, 42%, and 24% for 1, 2, 4, 8 and 16 Hz, respectively) compared with 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 with 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 nitrergic 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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Key Words. Diabetes; Erectile Dysfunction; Nitric Oxide; Arginase

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 nondiabetic [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 [8–10]. Previous studies in our lab and others indicate that diabetes elevates vascular Arg activity, which contributes to endothelial dysfunction [11–13]. 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 (ACh) in hypertensive, high-fat diet and diabetic models [11,17,18], whereas overexpression 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]. As Arg is upregulated 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): nondiabetic 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 three injections). In the nondiabetic 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 8 weeks after treatment.

Functional Studies

After 8-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; $NaHCO_3$, 25; glucose, 5.6; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$ $7H_2O$, 1.17 and $CaCl_2$ $2H_2O$, 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 $11 \times 1 \times 1$ 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% O_2 and 5% CO_2. Isometric force was recorded using a PowerLab/8SP data acquisition system (Software Chart, version 5, AD Instrument, Colorado Springs, CO, USA). The tissues were allowed to equilibrate for 1 hour before starting the experiments.