Erectile Dysfunction in Young Non-Obese Type II Diabetic Goto-Kakizaki Rats is Associated with Decreased eNOS Phosphorylation at Ser[1177]

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ABSTRACT

Introduction. Diabetes mellitus (DM) is a risk factor for erectile dysfunction (ED). Although type 2 DM is responsible for 90–95% diabetes cases, type 1 DM experimental models are commonly used to study diabetes-associated ED.

Aim. Goto-Kakizaki (GK) rat model is relevant to ED studies since the great majority of patients with type 2 diabetes display mild deficits in glucose-stimulated insulin secretion, insulin resistance, and hyperglycemia. We hypothesized that GK rats display ED which is associated with decreased nitric oxide (NO) bioavailability.

Methods. Wistar and GK rats were used at 10 and 18 weeks of age. Changes in the ratio of intracavernosal pressure/mean arterial pressure (ICP/MAP) after electrical stimulation of cavernosal nerve were determined in vivo. Cavernosal contractility was induced by electrical field stimulation (EFS) and phenylephrine (PE). In addition, nonadrenergic-noncholinergic (NANC)- and sodium nitroprusside (SNP)-induced relaxation were determined. Cavernosal neuronal nitric oxide synthase (nNOS) and endothelial nitric oxide synthase (eNOS) mRNA and protein expression were also measured.

Main Outcome Measure. GK diabetic rats display ED associated with decreased cavernosal expression of eNOS protein.

Results. GK rats at 10 and 18 weeks demonstrated impaired erectile function represented by decreased ICP/MAP responses. Ten-week-old GK animals displayed increased PE responses and no changes in EFS-induced contraction. Conversely, contractile responses to EFS and PE were decreased in cavernosal tissue from GK rats at 18 weeks of age. Moreover, GK rats at 18 weeks of age displayed increased NANC-mediated relaxation, but not to SNP. In addition, ED was associated with decreased eNOS protein expression at both ages.

Conclusion. Although GK rats display ED, they exhibit changes in cavernosal reactivity that would facilitate erectile responses. These results are in contrast to those described in other experimental diabetes models. This may be due to compensatory mechanisms in cavernosal tissue to overcome restricted pre-penile arterial blood supply or impaired veno-occlusive mechanisms. **Carneiro FS, Giachini FRC, Carneiro ZN, Lima VV, Ergul A, Webb RC, and Tostes RC. Erectile dysfunction in young non-obese type II diabetic Goto-Kakizaki rats is associated with decreased eNOS phosphorylation at Ser[1177]. J Sex Med 2010;7:3620–3634.**

Key Words. Goto-Kakizaki; Type 2 Diabetes Mellitus; Erectile Dysfunction; Corpus Cavernosum

Introduction

Diabetes mellitus (DM) is a heterogeneous group of disorders characterized by high blood glucose levels. The pancreatic beta-cell and its secretory product, insulin, are central in the pathophysiology of diabetes [1,2]. Type 1 or insulin-dependent diabetes mellitus (T1DM) results from an absolute deficiency of insulin due to autoimmune beta-cell destruction. In type 2, non-insulin-dependent diabetes mellitus (T2DM), liver, muscle, and fat cells are resistant to the

actions of insulin. The compensatory attempt by the beta-cell to release more insulin is not sufficient to maintain blood glucose levels within a normal physiological range, finally leading to the functional exhaustion of the surviving beta-cells [3,4]. Despite genetic predisposition, the risk of developing type 2 diabetes in humans increases with age, obesity, cardiovascular disease (hypertension, dyslipidemia), and a lack of physical activity [5,6].

Numerous studies have demonstrated that patients with DM have high rates of erectile dysfunction (ED) [7–9], whose prevalence ranges from 20% to 71% [10–12]. Fedele and coworkers [10] reported the prevalence of ED to be 51% among individuals with T1DM and 37% among those with T2DM in a cohort of approximately 10,000 men. Moreover, the Massachusetts Male Aging Study noted that the prevalence of ED in diabetic men is 50.7 per 1,000 population-years vs. 24.8 in those without diabetes [13].

Investigations in animal models and diabetic patients have implicated several mechanisms responsible for diabetes-associated ED, such as impaired vasodilatory signaling [14], nonadrenergic-noncholinergic (NANC) dysfunction [14], endothelial dysfunction [15–17], oxidative stress [18], pro-inflammatory changes, cavernosal hypercontractility [17,19], veno-occlusive dysfunction [17], and hypogonadism [20].

In a recent review, Hidalgo-Tamola and Chitaley [21] emphasize that despite the fact that both T1DM and T2DM entail abnormal carbohydrate metabolism and hyperglycemia, these diseases differ in many characteristics including insulin and body mass index status as well as cytokine and lipid profiles. T2DM is responsible for 90–95% of cases of diabetes, although clinical and epidemiological studies seldom separate T1DM and T2DM. In addition, fewer than 10 basic science studies addressing mechanisms of ED in animal models of T2DM have been published.

The Goto-Kakizaki (GK) rat is a polygenic model of T2DM obtained through selective inbreeding of Wistar rats with abnormal glucose tolerance over several generations. It is characterized by non-obesity, moderate but stable fasting hyperglycemia, hypoinsulinemia, normolipidemia, impaired glucose tolerance, which appears at 2 weeks of age in all animals, and an early onset of diabetic complications. In adult GK rats, total pancreatic beta cell mass is decreased by 60% along with a similar decrease in pancreatic insulin stores. In addition to the defects in beta cells, impaired insulin sensitivity in the liver, skeletal muscle, and adipose tissues has also been reported. Impaired insulin secretion and hepatic glucose overproduction (hepatic insulin resistance) are early events in diabetic GK rats mostly contributing to development of hyperglycemia rather than the peripheral (muscle and adipose tissue) insulin resistance [22,23].

Although the GK rat is a valuable diabetes model that shares several features of the T2DM observed in humans, no studies on erectile function in these animals have been reported in the literature. Considering that: (i) diabetes induces ED; (ii) there are only few studies evaluating the effects of T2DM in erectile function [21]; (iii) nitric oxide (NO) plays a major role in erectile function, we have hypothesized that GK rats display ED which is associated with decreased NO bioavailability. To test our hypothesis, we have used GK rats of 10 and 18 weeks of age to determine erectile function and changes in corpora cavernosa reactivity to contractile and relaxant stimuli.

Methods

Animals

Male Wistar rats (10 and 18 weeks old; Harlan, Indianapolis, IN, USA) and GK rats (10 and 18 weeks old; in-house bred derived from the Tampa colony) were used in the present studies. All procedures were performed in accordance with the Guiding Principles in the Care and Use of Animals, approved by the Medical College of Georgia Committee on the Use of Animals in Research and Education. The animals were housed four per cage on a 12-hour light/dark cycle and fed a standard chow diet with water ad libitum.

Blood Glucose Measurement

Animals were fasted from 8 AM to 12 PM (5 hours). Blood was drawn from the tail vein and glucose was measured by a commercially available glucose meter (Accu-Chek Active, Indianapolis, IN, USA).

In Vivo Measurements of Intracavernosal Pressure/ Mean Arterial Pressure

Intracavernosal pressure (ICP) in response to electrical stimulation of the cavernosal nerve was assessed in control and GK rats, as previously described [24,25]. Animals were anaesthetized with 4% isoflurane in 10% oxygen (O_2). To monitor and calculate mean arterial pressure (MAP) and