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Changes in caveolin-1 expression and vasoreactivity in the aorta and corpus cavernosum of fructose and streptozotocin-induced diabetic rats

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ABSTRACT

Hyperglycemia is a common defining feature in the development of endothelial dysfunction which plays a key role in the pathogenesis of both type 1 and type 2 diabetes. Caveolin-1 is the main structural component of caveolae which might be involved in the pathophysiology of macrovascular complications of diabetes. In this study we aimed to observe the effect of caveolin-1 on functional responses of aorta and corpus cavernosum in the streptozotocin and fructose-induced diabetes groups. Type 1 diabetes was induced by intraperitoneal administration of streptozotocin (60 mg/kg),. Type 2 diabetes by adding fructose in the rat's drinking water (10% (w/v)) for 8 weeks. For insulin treatment; rats were treated with insulin (6 U/kg) for 8 weeks. In Type I and Type II diabetic groups the contractile responses of corpus cavernosum strips to phenylephrine (EC_{50}:1.82×10^{-5} M;1.47×10^{-5} M, respectively)and relaxation responses to acetylcholine (EC_{50}:7.5×10^{-5} M;4.48×10^{-5} M, respectively)were significantly impaired. Contractile responses of aortic-strips to phenylephrine in diabetic groups were markedly decreased (EC_{50}:$3.7\cdot10^{-7}$ M;$2.61\cdot10^{-7}$ M respectively) and dose-dependent relaxation responses to acetylcholine were also attenuated (EC_{50}:$3.23\cdot10^{-6}$ M; $2.0\cdot10^{-6}$ M respectively). Treatment with insulin improved the functional responses in the aorta and corpus cavernosum. Protein expression of caveolin-1 was increased in the aorta and corpus cavernosum of the diabetic groups, but this increase seen in the streptozotocin group was more significant than the fructose group. Our findings indicate that an attenuation of the functional responses in both diabetes groups were probably associated with an enhanced expression of caveolin-1, and therefore a decrease in the eNOS activity with a concomitant decrease in NO synthesis.

1. Introduction

Diabetes mellitus is a disorder with debilitating consequences and has a worldwide prevalence of 3–6% (Zimmet et al., 2001; Herman et al., 1984). Since both type 1 and type 2 diabetes mellitus leads to significant vasculopathy, this factor could contribute to the pathophysiologic basis of diabetic erectile dysfunction (Hecht et al., 2001; Sasaki et al., 2003; Vinik and Mehrabyan 2004). Erectile dysfunction develops in approximately 35–75% of the patients with diabetes mellitus (type1 and type2), which is about three times higher than that of the general population (Bacon et al., 2002; Romeo et al., 2000). The pathogenesis of diabetic vasculopaty and erectile dysfunction is not completely understood.

Caveolae are specialized plasma membrane invaginations on the surface of most cells including endothelial cells and smooth muscle cells. Caveolae are characterised by a group of structural proteins called the caveolins (Linder et al., 2006). The functions of caveolin-1 include cholesterol transport, caveolae formation, G protein subunit regulation, oncogenic transformation, insulin signaling and endothelial nitric oxide synthase (eNOS) regulation (Glenny 1992; Fielding and Fielding, 1995; Shaul and Anderson 1998; Predescu et al., 1998). Caveolin-1 has attracted much attention to its ability to interact with eNOS. The binding of eNOS to caveolin-1 inhibits eNOS activity which is responsible for NO production in the endothelium (Lam et al., 2006). Understanding the role played by caveolae/caveolin in eNOS regulation may reveal new pathogenic mechanisms linking signal transduction molecules localized in these microdomains to the decreased production of NO. Furthermore, it has been stated that strategies that reduce caveolin expression may have important therapeutic implications in augmentation of NO availability in cardiovascular diseases (Dhillon et al., 2003) and erectile dysfunction (Linder et al., 2005).

It has been suggested that increased expression of caveolin-1 in aorta rings could represent a new possible therapeutic target in

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vascular impairment associated with type 1 diabetes (Bucci et al., 2004). It has also been demonstrated that impairment of acetylcholine-induced aortic relaxation in insulin resistant + db/+db mice is associated with an enhanced expression of caveolin-1, further indication that caveolin-1 plays an important role in cardiovascular complications of type 2 diabetes (Lam et al., 2006). Based on the importance of caveolae in NO signaling, we hypothesize that caveolin expression will significantly effect the tissue contraction and relaxation both in type 1 and type 2 diabetes. The relation between contractile responses and caveolin-1 expression has been studied in the aortic tissue of streptozotocin-induced diabetes (type 1). On the other hand neither the effect of caveolin-1 on functional responses in the corporal tissue nor the mechanisms of the effect of caveolin-1 on the development of erectile dysfunction in fructose-induced diabetes (type 2) has not been studied. In the present study we aimed to examine and to compare the influence of diabetes on caveolin-1 expression and on contractility of aorta and corpus cavernosum in rat models of type1 and type 2 diabetes. In addition, we have evaluated the effect of insulin treatment in these diabetes models.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–300 g) were obtained from the animal facility of the Marmara University, Medical School Istanbul, Turkey. Animals were housed on a 12 h light–dark cycle and received food and water ad libitum. The study was approved by the Marmara University, School of Medicine, Animal Care and Use Committee.

2.2. Experimental design

The rats were divided into the following groups ($n = 6$/group): control (C), injected with saline (1 ml/kg, i.p.); diabetes (D), injected with streptozotocin (60 mg/kg,i.p.); diabetes + ınsulin (DI), injected with streptozotocin (60 mg/kg, i.p.) and administered insulin (6 U/kg, s.c.) daily for 8 weeks; fructose (F), received 10% (w/v) fructose in their drinking water for 8 weeks; and fructose + ınsulin (FI), received fructose in their drinking water for 8 weeks and administered insulin (6 U/kg, s.c.) daily starting at week 2.

2.3. Measurement of blood glucose and plasma ınsulin levels

Forty eight hours after streptozotocin injection and 2 weeks after fructose treatment, blood samples were taken from the retro-orbital venous plexus using a hematocrit capillary tube and blood glucose levels were determined using a glucometer (Glucotrend, Roche, Germany). Rats with blood glucose levels >11.1 mM were considered to be diabetic and were included in the study. At the end of the experimental period of 8 weeks, before sacrificing the animals, blood samples were taken for the measurement of insulin levels. After collecting the blood samples, serum was separated by centrifugation and stored at −20 °C until analysis. Serum insulin levels were measured using an ELISA kit that is highly specific for both mouse and rat insulin (sensitivity:39 pg/ml) (Crystal-chem,USA, product # INSKR020).

2.4. Tissue collection

At the end of 8 weeks, animals were decapitated and the penis and aorta were rapidly dissected for in vitro organ bath studies, stored at −80 °C for the western blot analysis of caveolin-1 and β-actin, or fixed in JEOL 1200 SX for electron microscopy.

2.5. In vitro organ bath experiments

The penis and thoracic aorta were carefully dissected and placed in a petri dish containing chilled Krebs–Henseleit buffer as described previously (Paskaloglu et al., 2004). For isolation of corpus cavernosum strips, the penis was pinned out in the petri dish and the urethra and dorsal vein were quickly removed. After removing the surrounding connective tissue, strips of corpus cavernosum, yielding about $2 \times 2 \times 15$ mm was obtained. For isolation of the aorta, the surrounding connective tissue was removed and aorta was cut transversely into approximately 4 mm wide ring segments. Corpus cavernosum strips or aorta rings were then mounted in 20 ml organ bath containing Krebs–Henseleit buffer (118.14 mM. NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 11.1 mM glucose), gassed with 95% O_2 and 5% CO_2, and maintained at 37 °C, at pH 7.4. Both the corpus cavernosum strips and aorta rings were placed under a resting tension of 1.0 g and allowed to equilibrate for 60 min. Isometric contractions were recorded using a model FT03 force-displacement transducer coupled to a model 7 polygraph (Grass Instruments, Quincy, MA, USA). Following the equilibration period, the corpus cavernosum and aorta were exposed to 124 mM and 80 mM KCl, respectively to check the viability of the tissues. In both the corpus cavernosum strips and aorta rings, the contractile responses to 10^{-8}–10^{-3} M of phenylephrine were determined cumulatively. Contractile responses to phenylephrine were expressed as the percentage of KCl-induced maximal contraction. After 30 min washout period, relaxation responses to acetylcholine (10^{-8}–10^{-3} M) and sodium nitroprusside (10^{-8}–10^{-3} M) were evaluated in tissues precontracted with the submaximal concentration of phenylephrine (30 μM) and expressed as percentage of phenylephrine-induced contraction.

2.6. Western blot

The aorta and penile tissues were homogenized in homogenization buffer in the presence of protease and phosphatase inhibitors to obtain extract of proteins, centrifuged (10 000 × *g*, 30 min) and supernatants were collected. Protein concentrations were determined using BCA TM protein assay kit (Pierce, Rockford USA). Samples (25 μg of total protein) were loaded onto 12% Tris–HCl gels (Bıo-Rad), seperated by electrophoresis (180 V, 60 min) and were transferred (80 mA, 120 min) to polyvinylidene difluoride membrane (PVDF). Membranes were incubated with 5% non-fat dry milk for 2 h to block non-specific binding, rinsed with tris buffered saline with 0.5% Tween 20 (TBS-T) and incubated with anti-caveolin-1 antibody (1:6000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-β actin antibody (1:5000 dilution, Sigma, MO, USA) overnight at 4 °C. Membranes were washed 3 times for 10 min in TBS-T and incubated in a 1:6000 dilution of the goat anti-mouse horseradish peroxidase (HRP, GE Healthcare UK Limited) conjugated secondary antibody for 1 h in TBS-T containing 5% non-fat dry milk. The binding of the specific antibody was visualized by exposing to the photographic film after treating with Western Lightning™ chemiluminiscence reagents (PerkinElmer™, Life ScienceInc.,Boston, MA, USA).

2.7. Electron microscopy

Corpus cavernosum and aorta segments were cut into small pieces and fixed in 2.5% phosphate-buffered glutaraldehyde (0.1 M, pH 7.2) overnight and postfixed in 1% phosphate-buffered osmium tetroxide (0.1 M, pH 7.2). The pieces were then dehydrated through graded concentration of ethanol, and embedded in Epon-812. One micron semi-thin sections were stained with toluidine blue. Ultrathin sections from selected blocks were stained with uranyl acetate and lead citrate and observed in a transmission electron microscope (JEOL 1200 SX). Histological assessments were made by