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Ischemic conditioning protects the rat retina in an experimental model of early type 2 diabetes

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ABSTRACT

Diabetic retinopathy is a leading cause of acquired blindness in adults, mostly affected by type 2 diabetes mellitus (T2DM). We have developed an experimental model of early T2DM in adult rats which mimics some features of human T2DM at its initial stages, and provokes significant retinal alterations. We investigated the effect of ischemic conditioning on retinal changes induced by the moderate metabolic derangement. For this purpose, adult male Wistar rats received a control diet or 30% sucrose in the drinking water, and 3 weeks after this treatment, animals were injected with vehicle or streptozotocin (STZ, 25 mg/kg). Retinal ischemia was induced by increasing intraocular pressure to 120 mmHg for 5 min; this maneuver started 3 weeks after vehicle or STZ injection and was weekly repeated in one eye, while control eyes were submitted to a sham procedure. Fasting and postprandial glycemia, and glucose, and insulin tolerance tests were analyzed. At 12 weeks of treatment, animals which received a sucrose-enriched diet and STZ showed significant differences in metabolic tests, as compared with control groups. Brief ischemia pulses in one eye and a sham procedure in the contralateral eye did not affect glucose metabolism in control or diabetic rats. Ischemic pulses reduced the decrease in the electroretinogram a-wave, b-wave, and oscillatory potential amplitude, and the increase in retinal lipid peroxidation, NOS activity, TNFα, Müller cells glial fibrillary acidic protein, and vascular endothelial growth factor levels observed in diabetic animals. In addition, ischemic conditioning prevented the decrease in retinal catalase activity induced by T2DM. These results indicate that induction of ischemic tolerance could constitute a fertile avenue for the development of new therapeutic strategies to treat diabetic retinopathy associated with T2DM.

Introduction

Diabetes mellitus is a worldwide growing disease and represents a huge social and healthcare problem owing to the burden of its complications. The incidence of type 2 diabetes mellitus (T2DM) has increased significantly in the past decades, and is expected to continue to rise (Shaw et al., 2010). Chronic hyperglycemia of diabetes leads to microvascular and macrovascular circulatory impairment that has a negative impact on several organs. Diabetic retinopathy (DR) may be the most common of diabetes complications, and is a leading cause of visual impairment and blindness in people of working age (Fong et al., 2004). Current treatments for DR such as laser photocoagulation, corticosteroids, or anti-vascular endothelial growth factor agents are not fully efficacious, and may have significant adverse effects. Therefore, the development of resources to protect the retina against diabetic damage is a goal of vast clinical importance.

Understanding the molecular mechanisms of retinal damage associated with T2DM should help identify therapies to treat/postpone this sight-threatening complication of diabetes. For this purpose, some genetically modified animal models including transgenic, generalized knockout, and tissue specific knockout mice have been employed. However, a typical diabetic profile is not always seen in these genetically induced models, nor do they absolutely mimic the pathogenesis of human T2DM (Matsuura et al., 2005; Movassat et al., 1995), since these gene mutations are extremely rare in human populations. In a similar way, animal models of T2DM induced by removal of a portion of the pancreas (Portha et al., 1989) are not representative of T2DM etiology in humans which is typically preceded by obesity (Bray, 2004; Goralski and Sinal, 2007). In order to better understand the events

Abbreviations: AUC, area under the curve; DR, diabetic retinopathy; ERG, electroretinogram; GCL, ganglion cell layer; GFAP, glial fibrillary acidic protein; IPC, ischemic preconditioning; IPGTT, intraperitoneal glucose tolerance; IPITT, intraperitoneal insulin tolerance test; MDA, malondialdehyde bis-dimethyl acetal; NOS, nitric oxide synthase; OPs, oscillatory potentials; PostC, ischemic postconditioning; STZ, streptozotocin; TBARS, thiobarbituric acid reactive substances; T2DM, type 2 diabetes mellitus; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor.

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which precede and precipitate the onset of T2DM, some nutritional animal models have been also developed (Mühlhausler, 2009; Surwit et al., 1988).

Initially, the natural history of T2DM includes a period of normal or near-normal fasting plasma glucose levels and marked postprandial glycemic excursions. Historically, it was believed that microvascular complications of diabetes including T2DM develop only after ~10 to 15 years of active disease (UKPDS Group, 1995, 1998). However, it is increasingly clear that complications may begin at lower glucose concentrations or during sporadic increases in glucose levels, rather than after current thresholds for the diagnosis of T2DM are consistently reached (Stolar, 2010). Recently, we have developed an experimental model of T2DM through a combination of diet-induced insulin resistance and a slight secretory impairment resulting from a low-dose streptozotocin (STZ) treatment. This model mimics some features of human T2DM at its initial stages, such as slight fasting hyperglycemia, hyperinsulinemia, and elevated postprandial (nocturnal) glycemic levels (Salido et al., 2012). Noteworthy, only animals exposed to this combined treatment develop significant retinal alterations, whereas each one of these maneuvers *per se* (a sucrose-enriched diet or the injection of a low dose of STZ) does not induce significant retinal changes (Salido et al., 2012). Thus, this model could offer the opportunity to investigate DR at an early stage in a setting of moderately altered glucose metabolism.

DR is the most common ischemic disorder of the retina (Stitt et al., 2011). Ischemic retinopathy develops when retinal blood flow is insufficient to match the metabolic needs of the retina, one of the highest oxygen-consuming tissues. Although at present there is no effective treatment against retinal ischemic injury, it is possible to activate an endogenous protection mechanism that protects from retinal ischemic damage by ischemic preconditioning (IPC) or ischemic postconditioning (PostC) (Fernandez et al., 2009; Roth et al., 1998). IPC and PostC require a brief period of ischemia applied before or after ischemic injury, respectively, which do not produce any damage *per se*, and trigger yet incompletely described mechanisms that result in tolerance to the subsequent or previous severely damaging ischemic event (Gidday, 2006). It was shown that IPC and PostC afford the retina a very high degree of protection against acute ischemic damage (Fernandez et al., 2009; Roth et al., 1998). In this context, the aim of the present work was to analyze the effect of brief ischemia pulses on retinal alterations observed in an experimental model in rats which mimics early stages of human T2DM.

Materials and methods

Animals

Male Wistar rats (400 ± 50 g) derived from stock supplied by Charles River Breeding Laboratories (Wilmington, MA, USA) were purchased from a local dealer. Rats were housed in a standard animal room with food and water *ad libitum* under controlled conditions of humidity, temperature (21 ± 2 °C), and luminosity (200 lux), under a 12-hour light/12-hour dark lighting schedule (lights on at 7:00 AM). All the experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. The ethic committee of the School of Medicine, University of Buenos Aires (Institutional Committee for the Care and Use of Laboratory Animals, (CICUAL)) approved this study.

Feeding and treatments

Experimental groups included in this study are depicted in Fig. 1. Rats were randomly assigned to receive either a standard commercial rat chow and tap water (control groups), or a standard commercial rat chow and 30% sucrose in the drinking water, and an i.p. injection of STZ (25 mg/kg in 0.1 M citrate buffer, pH 4.5) 3 weeks after receiving 30% sucrose in the drinking water (T2DM groups). Three weeks after vehicle or STZ-injection, weekly ischemia pulses were applied in one eye, whereas the contralateral eye was submitted to a sham procedure. For retinal ischemia induction, animals were anesthetized with ketamine hydrochloride (150 mg/kg) and xylazine hydrochloride (2 mg/kg) administered intraperitoneally. After topical instillation of proparacaine, the anterior chamber was cannulated with a 30-gauge needle connected to a pressurized bottle filled with sterile saline solution. Retinal ischemia was induced by increasing intraocular pressure (IOP) to 120 mm Hg for exactly 5-min, as previously described (Fernandez et al., 2011). With

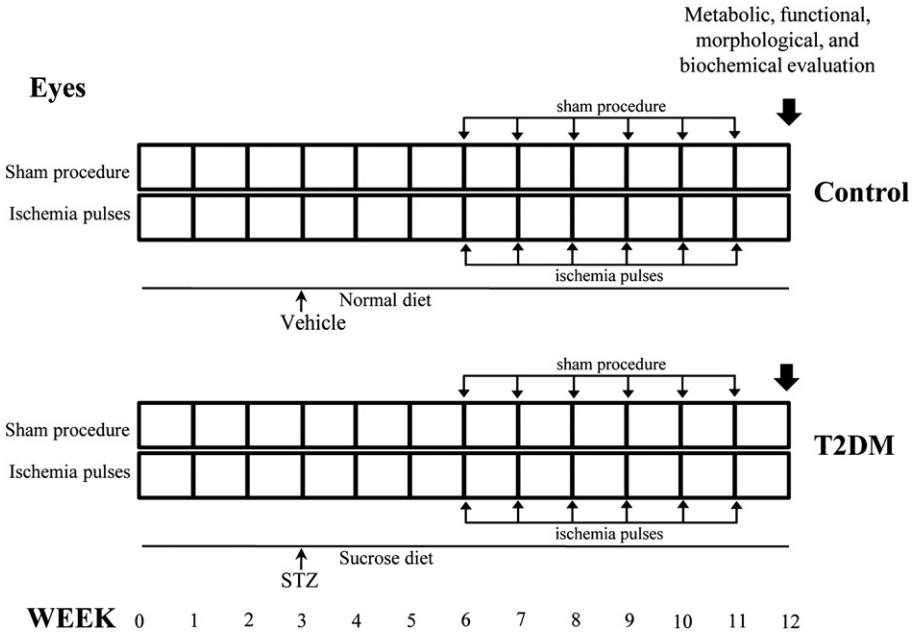

Fig. 1. Experimental groups for retinal studies. Animals were submitted to a normal diet (control) or a sucrose enriched diet (T2DM) for 12 weeks. At third week of diet, animals were i.p. injected with vehicle (control) or STZ (T2DM). Three weeks after vehicle or STZ-injection, animals received a 5-min ischemia pulse in one eye and a sham treatment in the contralateral eye. This maneuver was weekly repeated until week 11. For metabolic studies, groups of control and diabetic animals whose eyes remained intact were included. Retinal and metabolic studies were performed at 12 weeks of sucrose treatment.