Experimental Eye Research 127 (2014) 91–103

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Diabetes induces changes in KIF1A, KIF5B and dynein distribution in the rat retina: Implications for axonal transport

Filipa I. Baptista [a, b], Maria J. Pinto [c, d], Filipe Elvas [a, b], Tiago Martins [a, b], Ramiro D. Almeida [c], António F. Ambrósio [a, b, c, e, *]

[a] Centre of Ophthalmology and Vision Sciences, IBILI, Faculty of Medicine, University of Coimbra, 3004-548 Coimbra, Portugal
[b] Pharmacology and Experimental Therapeutics, IBILI, Faculty of Medicine, University of Coimbra, 3004-548 Coimbra, Portugal
[c] CNC-Center for Neuroscience and Cell Biology, University of Coimbra, 3004-517 Coimbra, Portugal
[d] PhD Programme in Experimental Biology and Biomedicine (PDBEB), Center for Neuroscience and Cell Biology, University of Coimbra, 3004-517 Coimbra, Portugal
[e] AIBILI, 3004-548 Coimbra, Portugal

ARTICLE INFO

Article history:
Received 1 March 2014
Accepted in revised form 15 July 2014
Available online 24 July 2014

Keywords:
diabetes
retina
axonal transport
kinesin
dynein

ABSTRACT

Diabetic retinopathy is a leading cause of vision loss and blindness. Disruption of axonal transport is associated with many neurodegenerative diseases and might also play a role in diabetes-associated disorders affecting nervous system. We investigated the impact of type 1 diabetes (2 and 8 weeks duration) on KIF1A, KIF5B and dynein motor proteins in the retina. Additionally, since hyperglycemia is considered the main trigger of diabetic complications, we investigated whether prolonged exposure to elevated glucose could affect the content and distribution of motor proteins in retinal cultures. The immunoreactivity of motor proteins was evaluated by immunohistochemistry in retinal sections and by immunoblotting in total retinal extracts from streptozotocin-induced diabetic and age-matched control animals. Primary retinal cultures were exposed to high glucose (30 mM) or mannitol (osmotic control; 24.5 mM plus 5.5 mM glucose), for seven days. Diabetes decreased the content of KIF1A at 8 weeks of diabetes as well as KIF1A immunoreactivity in the majority of retinal layers, except for the photoreceptor and outer nuclear layer. Changes in KIF5B immunoreactivity were also detected by immunohistochemistry in the retina at 8 weeks of diabetes, being increased at the photoreceptor and outer nuclear layer, and decreased in the ganglion cell layer. Regarding dynein immunoreactivity there was an increase in the ganglion cell layer after 8 weeks of diabetes. No changes were detected in retinal cultures. These alterations suggest that axonal transport may be impaired under diabetes, which might contribute to early signs of neural dysfunction in the retina of diabetic patients and animal models.

1. Introduction

Diabetic retinopathy is the most common microvascular complication of diabetes mellitus and is a leading cause of vision loss and blindness among working-age adults in Western countries. However, increasing evidence has shown that the neural components of the retina are also affected (Antonetti et al., 2006). Alterations in electroretinograms in diabetic patients and animals, and loss of color and contrast sensitivity are early signs of neural dysfunction in the retina (Roy et al., 1986; Daley et al., 1987; Sakai et al., 1995), demonstrating that the neural retina can be also affected by this disease.

Neurons are highly polarized cells, with long axons, which constitute a major challenge to the movement of proteins, vesicles, and organelles between cell bodies and presynaptic sites. To overcome this, neurons possess specialized transport machinery consisting of cytoskeletal motor proteins (kinesins and dynein) generating directed movements along cytoskeletal tracks. Axonal transport motor proteins require ATP demands, which implies the localization of functional mitochondria along the axons. Mobile mitochondria can become stationary or pause in regions that have a high metabolic demand and can move again rapidly in response to physiological changes. Defects in mitochondrial transport are implicated in the pathogenesis of several major neurological disorders (Sheng and Cai, 2012). Axonal transport is therefore crucial

* Corresponding author. Centre of Ophthalmology and Vision Sciences, IBILI, Faculty of Medicine, Azinhaga de Santa Comba, 3004-548 Coimbra, Portugal. Tel.: +351 239 480 093; fax: +351 239 480 280.
E-mail address: afambrosio@fmed.uc.pt (A.F. Ambrósio).

http://dx.doi.org/10.1016/j.exer.2014.07.011
0014-4835/

Abbreviations

ANOVA	analysis of variance
FBS	fetal bovine serum
GCL	ganglion cell layer
IL-1β	interleukin-1 beta
INL	inner nuclear layer
IPL	inner plexiform layer
NMDA	*N*-Methyl-D-aspartate
NO	nitric oxide
OCT	optimal cutting temperature gel
ONL	outer nuclear layer
OPL	outer plexiform layer
PBS	phosphate-buffered saline
PFA	paraformaldehyde
PHO	photoreceptor layer
RGC	retinal ganglion cells
RT	room temperature
STZ	streptozotocin
TBS	tris-buffered saline
TBS-T	tris-buffered saline containing Tween-20
TNF-α	tumor necrosis factor alpha
TUJ-1	neuron-specific class III beta-tubulin.

to maintain neuronal viability, and any impairment in this transport may play a role in the development or progression of several diseases (De Vos et al., 2008).

A decrease in the levels of mRNAs encoding for neurofilament proteins was found in the dorsal root ganglia of streptozocin-induced diabetic rats (Mohiuddin et al., 1995). Additionally, slow axonal transport of neurofilament and microtubule components is reduced, leading to a decrease in axonal caliber (Medori et al., 1988). These evidences suggest that deficits in axonal transport may contribute to neuronal changes observed in diabetes in neural tissues. To our knowledge, only a few studies have evaluated the effect of diabetes on axonal transport in the retina and most of them have focused in studying fluoro-gold labeling in retinal ganglion cells (RGCs) (Zhang et al., 1998; Ino-Ue et al., 2000; Zhang et al., 2000). Despite these evidences, the impact of diabetes in motor proteins (kinesins and dynein) in the retina has not been addressed. Nevertheless, potential changes in their content and distribution might underlie some changes already observed in axonal transport in the retina and visual pathway under diabetic conditions (Zhang et al., 2000; Fernandez et al., 2012).

Previously, we found that diabetes changes the levels of several synaptic proteins in retinal nerve terminals, with no changes in total retinal extracts, suggesting that axonal transport of those proteins may be impaired in diabetes (Gaspar et al., 2010a). Hyperglycemia is considered the main pathogenic factor for the development of diabetic complications. We found that high glucose leads to an accumulation of vesicular glutamate transporter-1, syntaxin-1 and synaptotagmin-1 at the cell body in hippocampal cell cultures, further suggesting that axonal transport of these proteins to nerve terminals might be affected under hyperglycemic conditions (Gaspar et al., 2010b). Recently, we showed that mRNA levels and the content of kinesin motor proteins are altered in the hippocampus of diabetic rats (Baptista et al., 2013). We also demonstrated that high glucose leads to changes in the immunoreactivity of motor proteins and synaptic proteins specifically in the axons of hippocampal neurons further suggesting that anterograde axonal transport may be impaired in the hippocampus (Baptista et al., 2013). These changes detected in the hippocampus of diabetic rats lead us to check whether similar changes could also be occurring in the retina under diabetes. Therefore, in this work, we aimed to study the effect of diabetes and also high glucose *per se* (prolonged exposure for 7 days), mimicking hyperglycemic conditions, on the content and distribution of the motor proteins KIF1A (kinesin that transports synaptic vesicle precursors), KIF5B (kinesin involved in mitochondrial transport and in the transport of synaptic vesicle precursors and membrane organelles) and dynein (motor protein for retrograde axonal transport) in diabetic animals and primary rat retinal cell cultures. Since motor proteins need ATP to carry cargoes along the axons, the distribution of mitochondria was also analyzed in retinal neural cell cultures exposed to high glucose.

2. Material and methods

2.1. Animals

Male Wistar rats (Charles River Laboratories), eight weeks-old, were randomly assigned to control or diabetic groups. All procedures were in agreement with the EU Directive 2010/63/EU for animal experiments. Diabetes was induced with a single intraperitoneal injection of streptozotocin (STZ; 65 mg/kg, freshly dissolved in 10 mM sodium citrate buffer, pH 4.5) (Sigma, St. Louis, MO, USA). Hyperglycemic status (blood glucose levels exceeding 250 mg/dl) was confirmed two days after STZ injection with a glucometer (Elite, Bayer, Portugal). Before sacrifice, rats were weighted and blood samples were collected for measurement of glucose. Diabetic rats and age-matched controls were anesthetized with halothane and then sacrificed, 2 and 8 weeks after the onset of diabetes.

2.2. Preparation of total retinal extracts

The eyes of diabetic and age-matched control animals were enucleated and placed in cold phosphate-buffered saline (PBS, in mM: 137 NaCl, 2.7 KCl, 10 Na_2HPO_4, 1.8 KH_2PO_4, pH 7.4, at 4 °C). Retinas were dissected and lysed in RIPA buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.5% DOC, 0.1% SDS, 1 mM DTT) supplemented with complete miniprotease inhibitor cocktail tablets and phosphatase inhibitors (10 mM NaF and 1 mM Na_3VO_4). Then, lysates were sonicated and centrifuged at 16,000 × *g* for 10 min at 4 °C. The supernatant was collected and stored at −80 °C until use.

2.3. Primary cultures of rat retinal neural cells

Retinal cell cultures were obtained from the retinas of 3–5 days-old Wistar rats as previously described (Santiago et al., 2006). Cells were plated at a density of 2.0×10^6 cells per cm^2 on poly-D-lysine substrate (0.1 mg/ml) and were maintained at 37 °C in a humidified incubator with 5% CO_2/air. The concentration of glucose in control conditions was 5 mM. After 2 days in culture, cells were incubated with 25 mM D-glucose (30 mM final concentration, with 5 mM from culture medium) or 25 mM D-mannitol (plus 5 mM glucose from culture medium), which was used as an osmotic control, and maintained for additional 7 days in culture (nine days in culture).