Experimental Neurology xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

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

Endoplasmic reticulum stress contributes to prediabetic peripheral neuropathy

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ARTICLE INFO

Article history:
Received 14 June 2012
Revised 29 October 2012
Accepted 2 November 2012
Available online xxxx

Keywords:
Diabetic peripheral neuropathy
Endoplasmic reticulum stress
Eukaryotic initiation factor-2α
High-fat diet fed mouse
Motor nerve conduction velocity
Prediabetic peripheral neuropathy
Salubrinal
Sensory nerve conduction velocity
Streptozotocin
Trimethylamine oxide
Unfolded protein response
Zucker (*fa*/fa) rat

ABSTRACT

Growing evidence suggests that prediabetes and metabolic syndrome are associated with increased risk for the development of microvascular complications including retinopathy, nephropathy, and, most commonly, peripheral painful neuropathy and/or autonomic neuropathy. The etiology of these disabling neuropathies is unclear, and several clinical and experimental studies implicated obesity, impaired fasting glycemia/impaired glucose tolerance, elevated triglyceride and non-esterified fatty acids, as well as oxidative–nitrative stress. Endoplasmic reticulum stress resulting from abnormal folding of newly synthesized proteins and leading to the impairment of metabolism, transcriptional regulation, and gene expression, is emerging as a key mechanism of metabolic diseases including obesity and diabetes. We evaluated the role for this phenomenon in prediabetic neuropathy using two animal models i.e., Zucker (*fa*/*fa*) rats and high-fat diet fed mice which displayed obesity and impaired glucose tolerance in the absence of overt hyperglycemia. Endoplasmic reticulum stress manifest in upregulation of the glucose-regulated proteins BiP/GRP78 and GRP94 of unfolded protein response was identified in the sciatic nerve of Zucker rats. A chemical chaperone, trimethylamine oxide, blunted endoplasmic reticulum stress and alleviated sensory nerve conduction velocity deficit, thermal and mechanical hypoalgesia, and tactile allodynia. A selective inhibitor of eukaryotic initiation factor-2α dephosphorylation, salubrinal, improved glucose intolerance and alleviated peripheral nerve dysfunction in high-fat diet fed mice. Our findings suggest an important role of endoplasmic reticulum stress in the neurobiology of prediabetic peripheral neuropathy, and identify a new therapeutic target.

Introduction

Diabetic peripheral neuropathy (DPN) affects at least 50% of patients with both Type 1 and Type 2 diabetes, and is a leading cause of foot amputation (Boulton et al., 2005; Sinnreich et al., 2005; Tesfaye et al., 2010; Veves et al., 2008). Several groups reported a higher incidence of diabetes-like neuropathic changes in human subjects with impaired glucose tolerance (Smith et al., 2001, 2006; Sumner et al., 2003; Ziegler et al., 2009) and metabolic syndrome (Bonadonna et al., 2006; Costa et al., 2004; Isomaa et al., 2001; Pittenger et al., 2005; Smith et al., 2008), although the existence of an association between impaired fasting glucose or impaired glucose tolerance and neuropathy is not uniformly accepted (Dyck et al., 2007). The etiology of neuropathy developing prior to the overt hyperglycemia is not well understood, and a number of clinical and experimental studies implicate obesity, impaired fasting glycemia/impaired glucose tolerance, elevated triglyceride, cholesterol, and non-esterified fatty acids, as well as oxidative–nitrative stress (Coppey et al., 2011; Costa et al., 2004; Lupachyk et al., 2012; Obrosova et al., 2007; Oltman et al., 2005, 2008; Smith et al., 2006, 2008; Sumner et al., 2003; Vincent et al., 2009; Watcho et al., 2010; Ziegler et al., 2009).

Endoplasmic reticulum (ER) stress is emerging as an important mechanism of metabolic diseases including obesity and diabetes (Eizirik et al., 2008; Fu et al., 2011, 2012; Hummasti and Hotamisligil, 2010; Kars et al., 2010; Kharroubi et al., 2004; Maris et al., 2012). ER stress results from damage to ER, an organelle playing a pivotal role in the folding and processing of newly synthesized proteins. ER stress leads to aberrant transcriptional regulation and gene expression, ion channel failure, dysmetabolism, impaired signaling, oxidative stress, and inflammation (Eizirik et al., 2008; Hotamisligil, 2010a,b). To counteract ER stress, the ER mounts the unfolded protein response (UPR). Three canonical arms of UPR include 1) PKR-like eukaryotic initiation factor 2A kinase (PERK) which phosphorylates eukaryotic initiation factor-2α (eIF2α) to suppress general protein translation; 2) inositol-requiring enzyme-1 (IRE1) involved in recruitment of several signaling molecules, splicing and production of an active transcription factor called X box-binding protein 1 (XBP-1), ER chaperones such as glucose-regulated protein BiP/GRP78 (BiP) and glucose-regulated protein 94 (GRP94), as well as CCAAT/enhancer-binding protein homologous protein (CHOP) and other components of the ER-associated degradation process; and 3) activating transcription factor-6 (ATP-6) which translocates to the Golgi apparatus and produces there an active transcription factor ATP-6N stimulating expression of chaperones and XBP-1. These three canonical arms of the UPR act together to reduce general protein synthesis, facilitate protein degradation, and increase folding capacity to resolve ER stress (Eizirik et al., 2008; Hotamisligil, 2010a,b).

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http://dx.doi.org/10.1016/j.expneurol.2012.11.001

However, the excessive and long-term upregulation of UPR, and, in particular, XBP-1, CHOP, ATF-4, has been shown to result in cell injury (Eizirik et al., 2008; Hotamisligil, 2010a,b).

Recent reports implicate ER stress in the development of chronic diabetic complications such as nephropathy (Wu et al., 2010), early retinopathy (Zhong et al., 2012), as well as cognitive decline (Sims-Robinson et al., 2012). In the present study, we evaluated the role for ER stress in neuropathic changes associated with prediabetes and obesity. We used a pharmacological approach with two agents, a non-specific chemical chaperone and protein stabilizer, trimethylamine oxide (TMAO), counteracting ER stress in toto, and the specific inhibitor of eukaryotic initiation factor-2α (eIF2α) dephosphorylation, salubrinal (Boyce et al., 2005).

Materials and methods

Reagents

Unless otherwise stated, all chemicals were of reagent-grade quality, and were purchased from Sigma-Aldrich Chemical Co., St. Louis, MO. Salubrinal, a selective inhibitor of eIF2α dephoshorylation (Boyce et al., 2005), was purchased from Santa Cruz Biotechnology, Santa Cruz, CA. For Western blot analysis, rabbit polyclonal anti-GRP78/BiP and anti-GRP94 antibodies and mouse monoclonal HRP-conjugated anti-β-actin antibody were obtained from Abcam, Cambridge, MA. Rabbit polyclonal anti-phospho-eIF2α (ser51) and anti-eIF2α antibodies were obtained from Cell Signaling, Danvers, MA.

Animals

Background

Exploration of the mechanisms of neuropathic changes preceding overt diabetes is complicated by the lack of animal models that develop prediabetes and obesity first and then spontaneously transit to overt diabetes. For this reason, the mechanisms underlying prediabetes per se as well as end-organ damage associated with this condition are studied in Zucker fatty (*fa/fa*) rats (Henriksen et al., 2011; Muellenbach et al., 2008; Oltman et al., 2005, 2008; Tong et al., 2010; Zhou et al., 1998) and high-fat diet (HFD) fed mice (Coppey et al., 2011; Longo et al., 2011; Shevalye et al., 2012b; Sparks et al., 2005; Zawalich et al., 1995) that maintain metabolic abnormalities characteristic for prediabetes i.e., hyperinsulinemia, impaired glucose tolerance in the absence of overt hyperglycemia, hypertriglyceridemia and/or increased non-esterified fatty acid abundance, as well as hypercholesterolemia, during their whole life span. Both models exhibit nerve conduction deficit, small sensory nerve fiber dysfunction, and biochemical abnormalities in the peripheral nerve, spinal cord, and *vasa nervorum* (Coppey et al., 2011; Lupachyk et al., 2012; Obrosova et al., 2007; Oltman et al., 2005, 2008; Vincent et al., 2009; Watcho et al., 2010), and are, therefore, suitable for dissection of relative contribution of these phenomena to peripheral neuropathy in prediabetes. The experiments were performed in accordance with regulations specified by the Guide for the Care and Handling of Laboratory Animals (National Institutes of Health publication 85-23) and Pennington Biomedical Research Center Protocol for Animal Studies. To reduce the number of animals in our studies, the TMAO-treated Zucker fatty and Zucker lean rats described below in Experiment 1 and acipimox-treated Zucker fatty and Zucker lean rats (Lupachyk et al., 2012) were compared to the same untreated controls. The six groups (four treated and two untreated) were studied in the same experiment. The blood chemistry and nerve function data for these untreated Zucker fatty and Zucker lean rats have been published previously (Lupachyk et al., 2012). In a similar fashion, a part of the C57Bl6/J mice fed with HFD for 16 weeks in our nephropathy-related study (Shevalye et al., 2012a) were treated with salubrinal as described herein (experiment 2 below). Initial body weights, blood glucose concentrations, and glucose tolerance data in C57Bl6/J mice fed with normal chow or HFD for 16 weeks were published previously (Shevalye et al., 2012a).

Experiment 1

Zucker fatty and Zucker lean rats were purchased from Charles River, Wilmington, MA. They were fed a standard rat chow (PMI Nutrition International, Brentwood, MO) and had access to water ad libitum throughout the experiment. 16 week-old rats were weighed. Blood samples for glucose measurements were taken from the tail vein. Zucker fatty and Zucker lean rats were randomly divided into groups maintained with or without TMAO treatment, 110 mg kg^{-1} d^{-1}, in the drinking water, for another 4 weeks. Glucose tolerance test (2 g glucose, i.p., after 12-h fasting), and measurements of serum insulin, total cholesterol, VLDL/LDL cholesterol, triglyceride, and NEFA, as well as MNCV, SNCV, thermal and mechanical algesia, and tactile response thresholds were conducted in 16 week-old Zucker fatty and Zucker lean rats before TMAO treatment, and in 20 week-old untreated and TMAO-treated Zucker fatty and Zucker lean rats at the end of experiment.

Experiment 2

Mature male C57Bl6/J mice were purchased from Jackson Laboratories, Bar Harbor, ME, and had access to water ad libitum throughout the experiment. The mice were assigned to receive normal or high-fat diets (D12328, 10.5 kcal% fat, and D 12330, 58 kcal% fat with corn starch, respectively, Research Diets, Inc., New Brunswick, NJ), for 16 weeks. Then the mice were maintained with or without treatment with salubrinal for another 4 weeks. We used salubrinal at 1 mg kg^{-1} d^{-1}, i.e., the dose previously employed and shown effective in chronic studies in rodents (Pallet et al., 2008; Saxena et al., 2009; Wu et al., 2011; Zhu et al., 2008). Measurements of serum insulin, total cholesterol, triglyceride, and NEFA were performed at 16 weeks (prior to salubrinal administration) and at 20 weeks. Evaluation of MNCV, SNCV, thermal and mechanical algesia, and tactile response thresholds was performed at a baseline (prior to high-fat diet feeding), at 16 weeks (prior to salubrinal administration), and at 20 weeks.

Anesthesia, euthanasia and tissue sampling

At the end of both experiments, the animals were sedated by CO_2. Rats were immediately sacrificed by decapitation, and mice by cervical dislocation. Sciatic nerves (experiments 1 and 2) and spinal cords (experiment 2) were rapidly isolated, immediately frozen in liquid nitrogen, and stored at −80 °C prior to assessment of variables of UPR by Western blot analysis.

Serum insulin, lipids, and non-esterified fatty acids

Rat serum insulin concentrations were measured with the Ultra Sensitive Rat Insulin ELISA Kit from Crystal Chem, Downers Grove, IL, and mouse serum insulin concentrations with the Rat/Mouse Insulin ELISA Kit, Millipore, Billerica, MA. Rat and mouse serum total cholesterol concentrations were quantified with the Cholesterol Quantification Kit, MBL International, Woburn, MA, and rat and mouse serum triglyceride concentrations with the Triglyceride Quantification Kit, Abcam, Cambridge, MA. Rat VLDL/LDL concentrations were measured with the HDL and LDL/VLDL Cholesterol Assay Kit, Abcam, Cambridge, MA, and serum NEFA concentrations with the HR Series NEFA-HR(2) Kit, Wako Pure Chemical Industries, Osaka, Japan. All the measurements were performed according to the manufacturer's instructions.

Nerve functional studies

Nerve functional studies included measurements of sciatic motor nerve conduction velocity (MNCV) and hind-limb digital sensory

Please cite this article as: Lupachyk, S., et al., Endoplasmic reticulum stress contributes to prediabetic peripheral neuropathy, Exp. Neurol. (2013), http://dx.doi.org/10.1016/j.expneurol.2012.11.001