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Research paper

Upregulation of UCP2 in beta-cells confers partial protection against both oxidative stress and glucotoxicity

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ABSTRACT

Deterioration of pancreatic beta-cells plays a critical role in the development of type 2 diabetes. Among the various stressors contributing to these deleterious effects, glucotoxicity and superoxides have been proposed as major players. In this context, the mitochondrial uncoupling protein UCP2 is regularly associated with the stress response. In the present study, we tested the effects of UCP2 upregulation in mouse islets with beta-cell specific overexpression of UCP2 (RIP-*UCP2*). Islets were subjected to both chronic glucotoxicity (7 days at 30 mM glucose) and acute oxidative stress (200 μM H_2O_2 for 10 min). Increased UCP2 expression did not alter mitochondrial potential and ATP generation but protected against glucotoxic effects. Glucose-stimulated insulin secretion was altered by both glucotoxicity and oxidative stress, in particular through higher basal insulin release at non-stimulatory glucose concentrations. The secretory response to glucose stimulation was partially preserved in beta-cells overexpressing UCP2. The higher rate of cell death induced by chronic high glucose exposure was lower in RIP-*UCP2* islets. Finally, superoxide production was reduced by high glucose, both under acute and chronic conditions, and not modified by UCP2 overexpression. In conclusion, upregulation of UCP2 conferred protective effects to the stressed beta-cell through mechanisms not directly associated with superoxide production.

1. Introduction

Type 2 diabetes, characterized by hyperglycemia, develops as a consequence of significant loss of functional ß-cells secondary to chronic exposure to stressful pathophysiological conditions, such as high glucose [1–6]. Such conditions, recognized long ago and referred to as glucotoxicity [7–9], were then proposed to be mediated by oxidative stress that would be induced by the overload of glucose metabolism in the ß-cell [10–13]. Oxidative stress has been shown to directly disrupt metabolism-secretion coupling in insulin secreting cells [14–16]. However, the putative contribution of oxidative stress to the etiology of diabetes remains debated [17], as well as the contribution of high glucose to the generation of reactive oxygen species (ROS) [18–20].

Another controversy is associated with UCP2, a mitochondrial protein sharing 59% homology with the uncoupling protein UCP1 [21]. UCP2 was originally proposed to induce proton leakage dissipating the mitochondrial proton motive force [21]. Some studies reported that overexpression of UCP2 in insulin-secreting cells increases respiration [22] and limits ATP production and glucose-stimulated insulin secretion [23,24], while others observed no alteration of mitochondrial coupling [25,26]. Thus, different maneuvers changing UCP2 expression in pancreatic ß-cells reported by various groups did not raise a consensus on the function of UCP2, neither at the cellular level (reviewed in [27]) nor in animal models (reviewed in [28]). An extensive review of the available studies on UCP2 in the ß-cell highlighted the lack of evidence for a significant mitochondrial proton leak contributed by UCP2 [27]. Interestingly, a recent study has shown that UCP2 is in fact a mitochondrial C4-metabolite transporter [29]. Such a function could establish a dissipative proton circuit [27], compatible with some kind of mild uncoupling activity through the reverse transport of protons.

Whatever the exact function of UCP2, it has consistently been reported to be upregulated in endocrine cells as a stress response [15,30–34]. Therefore, UCP2 may confer some protection, although it is rather challenging to dissociate effects of induced UCP2 from other concomitant stress responses. Upregulation of UCP2 before exposure to the stressors is one way to address this question. This has been achieved previously using cytokines as stress inducer, showing that upregulation of UCP2 prevents further cytokine-induced ß-cell death through the suppression of ROS production [26].

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In the present study, we investigated the putative effects of upregulation of UCP2 in mouse ß-cells subjected to either chronic glucotoxicity or acute oxidative stress. In order to do so, we used pancreatic islets isolated from transgenic mice with ß-cell specific overexpression of *UCP2* (RIP-*UCP2*) exposed for 7 days to high glucose in the culture medium. We also studied the link between glucotoxicity and oxidative stress in general and the glucose contribution to ROS production in particular. The results show that high glucose impairs ß-cell function without promoting superoxide production and that UCP2 upregulation partially protects against both glucotoxicity and oxidative stress.

2. Material and methods

2.1. Animals and in vivo experiments

Generation and analysis of transgenic mice with ß-cell specific overexpression of *UCP2* (RIP-*UCP2*) have been described previously [26]. Mice from in-house breeding (CMU-zootechnie, Geneva, Switzerland) of 2–5 months of age were age-matched with C57BL/6 J (wild-type, WT) control mice. We followed the principles of laboratory animal care and the study was approved by the responsible ethics committee. Glucose tolerance tests were performed upon intraperitoneal injection of D-glucose (2 g/kg) after overnight fasting. Blood samples were taken at time 0 and 15 min and glucose levels were determined using Accu-Check Aviva glucometer (Roche Diagnostics). Insulin, glucagon, and leptin were measured using Luminex xMAP™ technology and commercially available kits (Bio-Plex Pro Diabetes Assays, Biorad and Milliplex Mouse Metabolic Magnetic Bead Panel, Millipore).

2.2. Isolation and culture of pancreatic islets

Pancreatic islets were isolated from WT and RIP-*UCP2* mice matched for sex and age by collagenase digestion followed by histopaque-10771 (Sigma-Aldrich, St.-Louis, MO) gradient centrifugation as described before [35]. Isolated islets were cultured overnight free-floating in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10 mM HEPES, 10% (v/v) heat-inactivated fetal calf serum, 100 U/mL penicillin, 100 µg/mL streptomycin; then hand-picked for experiments [35].

2.3. Glucotoxicity induction

Glucotoxicity was induced by culturing isolated islets at 30 mM glucose (glucotoxicity), compared with standard 11 mM (control), for 7 days at 37 °C in the presence of 5% CO_2 in serum-free RPMI-1640 medium containing 5 g/L bovine serum albumin (BSA, Sigma-Aldrich). The 11 mM glucose condition served as control since, unlike human islets, rodent islets are commonly cultured at this concentration and because it corresponds to non-fasting euglycemia (200 mg/dL = 11 mM) for most mouse strains [36]. Medium was refreshed every 2 days to restore consumed glucose.

2.4. Oxidative stress induction

Oxidative stress (ox-stress) was induced by transiently challenging WT and RIP-*UCP2* islets with H_2O_2 as described previously [14,15]. Briefly, cultured islets were maintained for 45 min in 2.8 mM glucose before exposure to a single acute oxidative stress (200 µM H_2O_2, Sigma-Aldrich), which was neutralized after 10 min by adding catalase (100units/mL, Sigma-Aldrich) to the medium. Islets were then washed in the same medium and either immediately collected after the ox-stress for immunoblotting and secretion assay or further cultured in standard RPMI-1640 medium (11 mM glucose) for a 3-day recovery period before experiments.

2.5. Immunoblotting

Protein extracts from mouse islets treated as described were subjected to electrophoresis on a 12% polyacrylamide gel, electro-transferred onto nitrocellulose membrane, and blocked with 3% BSA in PBS (1.4 mM KH_2PO_4, 8 mM Na_2HPO_4, 140 mM NaCl and 2.7 mM KCl at pH 7.3). Membranes were then incubated overnight at 4 °C with different antibodies: goat anti-human polyclonal antibody to UCP2 (1:1000, #6527, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and mouse monoclonal anti-actin (1:5000, #4700, Sigma-Aldrich) in PBS containing 3% BSA and 0.05% Tween-20. After washing 3 times with PBS supplemented with 0.05% Tween-20, membranes were incubated with a horseradish peroxidase (HRP)-conjugated donkey anti-goat (1:10000, #2056, Santa Cruz) or anti-mouse antibody (1:5000, NA931, Amersham Biosciences, UK) for 1hr at room temperature. After washes, the immunoreactivity was visualized by SuperSignal West Pico Chemiluminescent Substrate system (Pierce Biotechnology, Inc., Rockford, IL) and Molecular Imager ChemiDoc XRS system (Bio-Rad, Hercules, CA) controlled by Quantity One 1-D (Bio-Rad) analysis software.

2.6. Mitochondrial membrane potential

Following the culture period, islets from the different groups were maintained for 30 min at 2.8 mM glucose in KRBH buffer (KRBH, 135 mM NaCl, 3.6 mM KCl, 5 mM $NaHCO_3$, 0.5 mM NaH_2PO_4, 0.5 mM $MgCl_2$, 1.5 mM $CaCl_2$, and 10 mM HEPES at pH 7.4) containing 0.1% BSA (KRBH/BSA), washed, and then pre-incubated for 30 min in KRBH/BSA containing 2.8 mM glucose and 10 µg/mL rhodamine-123 (Life Technologies). The mitochondrial membrane potential was monitored at 37 °C in ImageXpress Micro Widefield High Content Screening system (Molecular Devices, Sunnyvale, CA) with excitation and emission filters set as 490 and 530 nm, respectively. During experiments, glucose was raised from low 2.8 mM to stimulatory 22.8 mM to induce hyperpolarization of the mitochondrial membrane. Complete mitochondrial membrane potential was revealed by the addition of 1 µM of the protonophore carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP, Sigma-Aldrich). Fluorescence intensity of single islet was recorded and analyzed with MetaXpress High Content Image Acquisition and Analysis Software 2.0 (Molecular Devices). Data were obtained from at least 3 independent islet preparations; normalized to signals obtained at low glucose before stimulation.

2.7. Cellular ATP generation

Cellular ATP levels were measured in pancreatic islets transduced with ATeam adenovirus expressing the fluorescence resonance energy transfer (FRET)-based ATP indicator [37]. Practically, on the 5th day of glucotoxicity induction, islets were transduced with ATeam adenovirus for 90 min in the presence of either 11 mM or 30 mM glucose and then maintained in corresponding culture condition for 36hr. Acquisition of ATeam fluorescence of islets was conducted within the next 12hr. Islets were kept at 2.8 mM glucose and then stimulated with 22.8 mM glucose, followed by addition of 2 mM azide. Data obtained from islets of at least 3 mice were analyzed as FRET signal (as assessed by the emission ratio YFP/CFP) and normalized to the azide response reflecting the mitochondrial contribution to ATP generation.

2.8. Cellular calcium levels

Cytosolic [Ca^{2+}] changes were monitored as ratiometric measurements of Fura-2 fluorescence. Isolated mouse islets were cultured on glass coverslips treated with poly-L-lysine (Sigma-Aldrich) and placed in a thermostatic chamber (Harvard Apparatus, Holliston, MA) before incubation with 2 µM Fura-2/acetoxymethyl ester (AM) for 60 min.