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Uncoupling protein 2 deficiency aggravates astrocytic endoplasmic reticulum stress and nod-like receptor protein 3 inflammasome activation

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ABSTRACT

Astrocytes play crucial roles in determining the susceptibility to oxidative stress in the brain, and uncoupling protein 2 (UCP2) has been demonstrated to regulate reactive oxygen species (ROS) production. However, it is unclear whether UCP2 is expressed in astrocytes, and whether it participates in the regulation of astrocytic functions. Here we show that UCP2 knockout exacerbated dopaminergic neuron loss in a murine model of 1,2,3,6-methyl-phenyl-tetrahydropyridine (MPTP)–induced Parkinson's disease (PD), accompanied by overactivation of astrocytes. We further detected expression of UCP2 in primary cultures of mesencephalic astrocytes. UCP2 knockout increased intracellular ROS production and induced oxidative stress in response to l-methyl-4-phenylpyridinium (MPP^+) treatment. Subsequently, UCP2 deficiency exacerbated endoplasmic reticulum (ER) stress, as evidenced by the upregulations of C/EBP homologous protein (CHOP), cleavage of caspase-12, and aggravated neuroinflammation via the activation of nod-like receptor protein 3 (NLRP3) inflammasomes in astrocytes. Collectively, our study indicates that UCP2 expressed in astrocytes modulates ER stress and neuroinflammation, and is crucial for the survival of dopaminergic neuron in the pathogenesis of PD. These findings gives us insights into the potential of UCP2 as a novel therapeutic avenue for PD treatment.

1. Introduction

Uncoupling protein 2 (UCP2) is located in the inner membrane of mitochondria and was discovered because of its sequence homology to the brown fat UCP1 (Mattiasson and Sullivan, 2006). The primary function of UCP2 is recognized to be the translocation of protons from intermembrane space to the matrix of mitochondria. Accumulating evidence supports that UCP2 does not contribute to adaptive thermogenesis (Azzu and Brand, 2010; Brand and Esteves, 2005; Yonezawa et al., 2009), but participates in the control of mitochondria-derived reactive oxygen species (ROS) (Echtay et al., 2001; Mailloux and Harper, 2011; Pi and Collins, 2010). For example, UCP2 knockout increased free radical production of macrophages in mice, whereas overexpression of UCP2 in vitro (immortalized β-cells) and in vivo (neurons) is protective against oxidative damage (Andrews et al., 2005a; Arsenijevic et al., 2000). In addition, oxidative stress induced by H_2O_2, lipopolysaccharide and irradiation can activate transcription, translation, and protein activity of UCP2 (Arsenijevic et al., 2000; Emre et al., 2007; Rousset et al., 2006). Thus, the major physiological function of UCP2 is to attenuate mitochondrial production of ROS. Activation and/or upregulation of UCP2 may function as an adaptive response to oxidative stress.

A growing body of evidence indicates that mitochondrial oxidative stress is critical in the pathogenesis of neurodegenerative diseases, including Parkinson's disease (PD) (Bueler, 2009; Gao et al., 2003; Iravani et al., 2002; Knott et al., 2000; McGeer and McGeer, 2004; Van Laar and Berman, 2009). Oxidative stress may account for the nigral defect of complex I, which is highly vulnerable to oxidative damage. Inhibition of complex I leads to increased ROS formation in the pathogenesis of PD (Lemasters, 2007). It has been reported that UCP2 knockout increased the loss of nigral dopamine neurons in 1,2,3,6-methyl-phenyl-tetrahydropyridine/probenecid (MPTP/p) PD model mice because of increased ROS production (Andrews et al., 2005b). Excessive ROS not only induces oxidative injury but also results in endoplasmic reticulum (ER) stress. Increasing evidence demonstrates that ER stress, in conjunction with abnormal protein degradation, can contribute to the pathophysiology of PD (Egawa et al., 2011; Ryu et al., 2002). ER stress activates the unfolded protein response that induces ER-associated protein degradation as a self-protective mechanism, thereby leading to rescue or adaptive responses

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(Hara et al., 2011; Rao et al., 2004). Nevertheless, excessive ER stress long term may result in cell apoptosis or even necrosis. In addition to ER stress, production of numerous inflammatory factors, such as interleukin-1β (IL-1β), has been considered to play an important role in neurodegenerative disorders including PD (Teismann and Schulz, 2004; Wen et al., 2012). Emerging evidence indicates that accumulated ROS leads to glial inflammation, including the activation of nod-like receptor protein 3 (NLRP3) inflammasome, which modulates the production of mature IL-1β (Wen et al., 2012). However, it remains unknown whether UCP2 regulates ER stress and NLRP3 inflammasome-initiated inflammation by controlling ROS production in astrocytes.

Astrocytes are the primary site for modulation of redox homeostasis, and play crucial roles in determining susceptibility of neuron to oxidative stress in the brain (Simpson et al., 2010); it is unclear, however, whether UCP2 is expressed in astrocytes and regulates astrocytic functions. Therefore, the primary goal of the present study was to identify UCP2 expression in primary cultured astrocytes. Thereafter, UCP2 knockout mice were used to establish PD models to investigate whether UCP2 participates in the pathogenesis of PD via modulating astrocytic ER stress and NLRP3 inflammasome activation.

2. Methods

2.1. Animals and reagents

UCP2 knockout (KO) mice (on a C57/B6 background) were obtained from Prof. Zhang Chenyu (Zhang et al., 2001) (School of Life Science, Nanjing University). Mice were bred and maintained in the Animal Resource Centre of the Faculty of Medicine, Nanjing Medical University. Three-month-old male C57BL/6 mice were used as wild-type (WT) controls. The WT and KO mice were housed with free access to food and water in a room with an ambient temperature of 22 °C ± 2 °C and a 12:12-hour light/dark cycle. All animal procedures were performed according to National Institutes of Health Guide for the Care and Use of Animals and approved by the Institutional Animal Care and Use Committee.

2.2. Primary astrocyte cultures

Briefly, neonatal rats were killed by rapid decapitation, the midbrain was removed and separated from meninges and basal ganglia, and tissue was dissociated with 0.25% trypase (Amresco, Solon, OH) at 37 °C and terminated by Dulbecco's modified Eagle's medium (Gibco-BRL, Rockville, MD) supplemented with 10% fetal bovine serum (Sigma, St Louis, MO). After centrifugation at 1500 rpm for 5 minutes, the cell pellets were resuspended and plated on a poly-lysine–treated flask (Sigma, St Louis, MO). The cultures were maintained at 37 °C in a humidified 5% CO_2–95% air atmosphere.

2.3. Measurement of intracellular ROS generation

Formation of ROS was evaluated using 2′,7′-dichlorofluorescein diacetate (DCFH-DA; Sigma, St Louis, MO), a membrane-permeable probe de-esterified intracellularly. The non-fluorescent dye, which freely penetrates cells, was then hydrolyzed by intracellular esterases to DCFH and trapped within the cells. ROS oxidize DCFH to the highly fluorescent compound, dichlorofluorescein (DCF). Cells were loaded with DCFH-DA (50 μmol/L final concentration) in Dulbecco's modified Eagle's medium for 30 minutes in the dark and fixed by 4% formaldehyde. After rinsing cells twice with phosphate-buffered saline solution (137 mmol/L NaCl, 2.7 mmol/L KCl, 9.5 mmol/L $Na_2HPO_4.12H_2O$, and 1.5 mmol/L KH_2PO_4, pH 7.20), fluorescence was read at the excitation wavelength of 480 nm and the emission wavelength of 530 nm with a Nikon Optical TE2000-S inverted fluorescence microscope. The mean green fluorescence of every well was quantified by image analysis software (Simple PCI; Compix, Cranberry Township, PA).

2.4. Mitochondrial ROS measurement

Mitochondria-associated ROS levels were measured by staining cells with MitoSOX (Invitrogen) at 2.5 μmol/L for 30 minutes at 37 °C. Cells were then washed with PBS solution and resuspended in cold PBS solution containing 1% fetal bovine serum for fluorescence-activated cell sorter (FACS) analysis by flow cytometry.

2.5. Hoechst staining

To quantify apoptotic astrocytes, astrocytic monolayer was fixed and stained with Hoechst 33324 (Sigma, St Louis, MO). The morphological features of apoptosis (cell shrinkage, chromatin condensation, and fragmentation) were monitored by fluorescence microscopy (Olympus BX 60, Tokyo, Japan). At least 400 cells from 12 randomly selected fields per dish were counted, and each treatment was performed in triplicate.

2.6. Lactate dehydrogenase release assay

Lactate dehydrogenase (LDH) release in culture medium in the presence of MPP^+ (50 μmol/L) was measured using an LDH diagnostic kit (Promega, Madison, WI) according to the manufacturer's instructions. LDH activity was calculated by measuring absorbance at 492 nm. Results are expressed as percentage of LDH release in control cells.

2.7. MPTP-induced PD mouse model

Four-month-old male UCP2-KO and UCP2-WT mice were injected subcutaneously with 20 mg/kg MPTP (Sigma, St. Louis, MO) in saline solution and intraperitoneally with 250 mg/kg probenecid in DMSO every 3.5 days over a period of 5 weeks, and were killed 7 days after the last injection. Control mice were treated with saline solution only.

2.8. Tyrosine hydroxylase and glial fibrillary acidic protein immunostaining

After animals were perfused with 4% paraformaldehyde, brains were dissected out and maintained in 4% paraformaldehyde overnight. Brains were cryopreserved in 30% sucrose in phosphate-buffered saline solution and then stored at −70 °C until use. Sections (30 μm) were incubated overnight with rabbit mouse monoclonal anti-TH antibody (1:3000; Sigma, St Louis, MO), or mouse anti–glial fibrillary acidic protein (GFAP) antibody (1:300; Chemicon) for the detection of tyrosine hydroxylase (TH) and GFAP, respectively, and then for 1 hour with secondary antibodies. Immunoreactivity was visualized by incubation in DAB. Control staining was performed without primary antibodies. The total numbers of TH-positive neurons and GFAP-positive and MAC-1–positive cell numbers in the SNc and ventral tegmental area (VTA) were obtained stereologically using the optical fractionator method (West, 1993).

2.9. In situ detection of ROS

Dihydroethidium (Molecular Probes, Eugene, OR) was used to investigate the local in situ production of total ROS. An