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Metabolic inflammation exacerbates dopaminergic neuronal degeneration in response to acute MPTP challenge in type 2 diabetes mice

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ABSTRACT

Parkinson's disease (PD), one of the most common neurodegenerative diseases, is characterized by the loss of dopaminergic neurons in the substantia nigra. Increasing epidemiological evidence has indicated that type 2 diabetes (T2D) may be implicated in the pathogenesis of PD. However, the exact association and the underlying mechanism remain unclear. In the present study, *ob*/*ob* and *db*/*db* mice, the well accepted T2D models, were acutely treated with MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) to mimic PD-like neural injury. We found that insulin signaling impairment occurred not only in pancreas and livers, but also in the midbrain of *ob*/*ob* and *db*/*db* mice. Notably, the expressions of monomeric and oligomeric α-synuclein as well as endoplasmic reticulum stress markers (CHOP and GRP78) were significantly upregulated in both pancreas and midbrain of T2D mice, accompanied by the increased activation of NLRP3 inflammasomes to produce excess IL-1β. Furthermore, we found that acute MPTP administration aggravated the loss of dopaminergic neurons and increased the activation of glial cells in the substantia nigra of *db*/*db* mice. Collectively, these findings demonstrate that α-synuclein accumulation and neuroinflammation are aggravated in the midbrain of T2D mice and T2D mice are more susceptible to the neurotoxicity induced by MPTP. Our study indicates that metabolic inflammation exacerbates DA neuronal degeneration in the progress of PD, which will provide a novel insight into the etiology of PD.

Introduction

Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder after Alzheimer's disease (AD) with a prevalence of 0.5–1% among people older than 65 years of age (Toulouse and Sullivan, 2008). PD is characterized clinically by a classic tetrad of motor symptoms: low-frequency resting tremor, rigidity of the skeletal muscles of the face and hands, reduced motor activity (bradykinesia), and in later stages of the disorder, postural instability (Corti et al., 2011). The cardinal symptoms of PD result mainly from the loss of dopaminergic neurons in the substantia nigra pars compacta (Hirsch et al., 1999) and formation of intraneuronal protein aggregates called Lewy bodies (Spillantini et al., 1997). However, these motor dysfunctions do not become apparent until 70–80% of nigrostriatal nerve terminals have undergone degeneration, suggesting the existence of an impressive compensatory mechanism in the earlier stages of the disease (Bernheimer et al., 1973).

Although PD has been emphatically investigated in the last two decades, the precise etiology of the disease is still unclear. Currently, a cluster of metabolic factors had been recognized to increase the risk of neurodegeneration as well as atherosclerotic cardiovascular disease. This cluster is regarded as the danger signal for "metabolic syndrome" and afflicted individuals are defined by the presence of three of the following five characteristics: central obesity, hypertriglyceridemia, low high-density lipoprotein (HDL)-cholesterol levels, hypertension, and either impaired fasting glucose or type 2 diabetes (Ginsberg et al., 2006). Type 2 diabetes (T2D), the most common type of diabetes, characterized by a reduction in the ability of insulin to stimulate glucose utilization, insulin resistance and inadequate pancreatic β-cell insulin secretion in response to hyperglycemia, is associated with a faster rate in the decline of cognition in comparison with the general population (Lu and Hu, 2012). Evidence from prospective epidemiological studies has identified type 2 diabetes as an independent risk factor for multiple complications virtually in all organs, including neurodegenerative diseases such as diabetic neuropathy (Boulton et al., 2005), stroke (Ergul et al., 2012; Ginsberg et al., 2006; Hu et al., 2006; Lawes et al., 2004; Schernhammer et al., 2011), and Alzheimer's disease (Corti et al., 2011; Hirsch et al., 1999).

Recently, increasing epidemiological researches focused on the relationship between diabetes and the risk of PD (Lu and Hu, 2012; Santiago and Potashkin, 2013). Gang hu et al. prospectively followed 51,552 Finnish men and women 25–74 years of age without a history of PD at baseline and they found that type 2 diabetes is associated with an increased risk of PD (Hu et al., 2007). Qun Xu et al. supported this view with their data that PD risk was 40% higher among diabetic patients

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than among participants without diabetes within a total of 1565 participants with PD diagnosed after 1995 were included in the analysis (Xu et al., 2011). At about the same time, Eva Schernhammer proposed that there is the common physiological pathway between PD and type 2 diabetes (Schernhammer et al., 2011). All the epidemiological results above indicate that there is a potential connection existing between PD and diabetes. However, the detailed mechanism underlying the association of PD and diabetes remains unknown.

In the present study, the type 2 diabetes mice were used to investigate the role of systemic metabolic inflammation in the pathogenesis of PD. Our data revealed that T2D mice exhibited the accumulation of α-synuclein, subsequent endoplasmic reticulum (ER) stress and NLRP3 inflammasome activation in the midbrain. Furthermore, inflammatory harms in T2D mice aggravated the vulnerability of dopaminergic neurons in response to MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) acute challenge, indicating that metabolic inflammation plays a key role in the pathogenesis of PD.

Materials and methods

Animals and treatment

Male C57BL/6J-*db*/*db* mice (*db*/*db* mice) and male C57BL/6J-*ob*/*ob* mice (*ob*/*ob* mice) aged 2.5 months were obtained from Mutant Mice Model Animal Research Center of Nanjing University (Nanjing, China). Male C57BL/6J mice were used as wild-type controls. All mice were bred and maintained in the Animal Resource Centre of the Faculty of Medicine, Nanjing Medical University, with free access to standard chow and water in a room with an ambient temperature of 22 ± 1 °C and a 12:12 h light/dark cycle. All experiments were carried out in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (publication no. 85–23, revised 1985) and all animals were maintained under specific pathogen-free conditions and treated according to protocols approved by IACUC (Institutional Animal Care and Use committee of Nanjing Medical University).

The acute MPTP intoxication protocol is similar to that described previously (Jackson-Lewis and Przedborski, 2007): Animals (n = 6) received four i.p. injections of 20 mg/kg MPTP (Sigma, St. Louis, MO) at 2 h intervals within a single day, for a cumulative dose of 80 mg/kg. Control mice received saline injections only. Animals were sacrificed at 3.5 days after the last injection of MPTP or saline.

Histochemistry

Liver and pancreas were removed from mice and fixed in 10% formalin, embedded in paraffin, and then sectioned at a thickness of 5 μm, followed by hematoxylin–eosin (H–E) staining. At the end of preparation of acute MPTP model, mice were perfused with 4% paraformaldehyde (PFA). Brains were dissected from the skull, postfixed overnight in buffered 4% PFA at 4 °C, stored in a 20% sucrose solution at 4 °C for 3 days, and then changed into 30% sucrose solution until they sank. The sections were permeabilized with PBS + 0.3%Triton X-100 for 10 min and blocked with 10% goat serum in PBS for 30 min and then incubated with primary antibody (mouse anti-TH, T1299; Sigma-Aldrich, St. Louis, MO, USA; mouse anti-GFAP, MAB 360, Millipore, NC, USA; rabbit anti-Iba-1, Wako Pure Chemical Industries, Osaka, Japan) overnight at 4 °C and appropriate secondary antibody for 1 h. Immunoreactivity was visualized by incubation in DAB. The immunostaining signals were quantitatively analyzed using the Optical Fractionator method with Microbrightfield Stereo-Investigator software (Stereo Investigator software; Microbrightfield).

Western blotting analysis

Cell fractionation and Western blotting were performed as described previously (Yang et al., 2012). The membranes were incubated with various primary antibodies overnight at 4 °C. The primary antibodies were rabbit anti-IRS-1 (21223, Signalway Antibody, Nanjing, China), rabbit anti-phospho-IRS-1^{ser636} (11230, Signalway Antibody, Nanjing, China), rabbit anti-Akt (21054, Signalway Antibody, Nanjing, China), rabbit anti-phospho-Akt^{ser473} (11054, Signalway Antibody, Nanjing, China), rabbit anti-GSK3β (21301, Signalway Antibody, Nanjing, China), rabbit anti-phospho-GSK3β^{Tyr216} (11301, Signalway Antibody, Nanjing, China), rabbit anti-ERK1/2 (5013, Cell Signaling Technology, Beverly, MA, USA), rabbit anti-phospho-ERK1/2 (Thr202/Tyr204) (4370S, Cell Signaling Technology, Beverly, MA, USA), goat anti-NLRP3 (sc-34410, Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-caspase-1 (sc-514, Santa Cruz Biotechnology, Santa Cruz, CA, USA), goat anti-IL-1β (AF-401-NA, R&D Systems, Minneapolis, MN, USA), rabbit anti-GRP78 (3183, Cell Signaling Technology, Beverly, MA, USA), mouse anti-chop (2895, Cell Signaling Technology, Beverly, MA, USA) and mouse anti-α-synuclein (610787, BD Transduction Laboratories, San Jose, CA, USA). The membrane was washed and incubated for 1 h at room temperature with the corresponding secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The bands were detected with HRP-conjugated secondary antibodies and visualized by enhanced chemiluminescence ECL (Pierce, Rockford, IL, USA). β-Actin was used as an internal control. The membranes were scanned and analyzed using ImageQuant™ LAS 4000 imaging system (GE Healthcare, Piscataway, NJ, USA).

Preparation of Triton X-100 soluble and insoluble fractions of α-synuclein

The aggregation state of α-synuclein was analyzed based on its differential solubility in 1% Triton X-100, as described previously (Zhou and Freed, 2004). Briefly, tissue homogenates were extracted in solubilization buffer (20 mM Tris–HCl, 50 mM NaCl, 1% Triton X-100, pH 7.4) with Complete Mini Protease Inhibitor Cocktail (Roche) and Halt Phosphatase Inhibitor Cocktail (Thermo Scientific). Lysates were incubated for 30 min on ice, followed by centrifugation at 15,000 ×*g* for 60 min at 4 °C and supernatant was collected as the Triton X-100-soluble fraction. The Triton X-100-insoluble pellets were re-dissolved in the same volume of lysis buffer containing 2% SDS then analyzed by immunoblot.

Enzyme linked immunosorbent assay

The levels of insulin, IL-1β, IL-6, IL-18, and TNF-α in serum of mice (R&D Systems, Minneapolis, MN, USA) were determined by ELISA according to the protocol of the manufacturer. Absolute cytokine concentrations were determined by comparison to a standard curve with a Varioskan Flash spectral scanning multimode reader (Thermo Fisher Scientific, Waltham, MA, USA).

Quantitative realtime PCR

Total RNA was extracted from midbrain using TriPure reagent (ROCHE, Diagnostics Ltd., Indianapolis, IN, USA) followed by treatment with RNase-free DNaseI (Invitrogen Life Technologies). Total RNA (2 μg) of each sample was reverse-transcribed into cDNA and amplified using a PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara, TaKaRa Biotechnology, Dalian, China) according to the manufacturer's directions. Real-time PCR was performed with Fast Start Universal SYBR Green Master (Rox) (Roche Diagnostics Ltd., Diagnostics Ltd., Indianapolis, IN, USA). Reactions were amplified and analyzed by mean of an ABI 7300 Real Time PCR System (Applied Biosystems Japan, Co. Ltd.). Each experiment was repeated 4 times.

Statistical analysis

Data are presented as the mean ± SEM. The significance of the difference with different treatments and genotypes was determined by