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Early-onset motor impairment and increased accumulation of phosphorylated α-synuclein in the motor cortex of normal aging mice are ameliorated by coenzyme Q

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ABSTRACT

Brain mitochondrial function declines with age; however, the accompanying behavioral and histological alterations that are characteristic of Parkinson's disease (PD) are poorly understood. We found that the mitochondrial oxygen consumption rate (OCR) and coenzyme Q (CoQ) content were reduced in aged (15-month-old) male mice compared to those in young (6-month-old) male mice. Concomitantly, motor functions, including the rate of movement and exploratory and voluntary motor activities, were significantly reduced in the aged mice compared to the young mice. In the motor cortex of the aged mouse brain, the accumulation of α-synuclein (α-syn) phosphorylated at serine129 (Ser129) significantly increased, and the level of vesicular glutamate transporter 1 (VGluT1) decreased compared with that in the young mouse brain. The administration of exogenous water-soluble CoQ_{10} to aged mice via drinking water restored the mitochondrial OCR, motor function, and phosphorylated α-syn and VGluT1 levels in the motor cortex. These results suggest that early-onset motor impairment and the increased accumulation of Ser129-phosphorylated α-syn in the motor cortex are ameliorated by the exogenous administration of CoQ_{10}.

1. Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative disorders and is characterized by the progressive degeneration of neurons, which is most profound in the substantia nigra pars compacta (SNc), and the presence of Lewy bodies (LBs), which are intracytoplasmic proteinaceous inclusions, in surviving neurons of the SNc and other mid-brain regions (Spillantini et al., 1997, 1998). Because LBs consist of fibrillar aggregates of the synaptic protein α-synuclein (α-syn) (Spillantini et al., 1997) and most of the protein deposited in LBs is phosphorylated at serine129 (Ser129) (Fujiwara et al., 2002; Anderson et al., 2006), the accumulation of abnormal α-syn, including Ser129-phosphorylated α-syn, is considered to cause neurodegeneration of the SNc in PD patients or mouse models of PD (Chen and Feany, 2005; Anderson et al., 2006; Sato et al., 2011, 2013) through brain mitochondria dysfunction (Li et al., 2007; Devi et al., 2008; Chinta et al., 2010).

Conversely, the mitochondrial dysfunction and oxidative stress associated with PD might also promote increased membrane permeability and cytochrome *c* release, which results in α-syn oligomerization and neurodegeneration (Büeler, 2009; Schon and Przedborski, 2011; Trancikova et al., 2012). These results suggest that the progressive impairment of mitochondrial activity plays a critical role in the pathogenesis of the neurodegenerative disorder of PD, and the accumulation of abnormal α-syn may be both a cause and an effect in the pathologies of brain mitochondria and neurodegenerative disorders.

A motor disability is another clinical feature of PD and is characterized by the cardinal motor symptoms of resting tremor, rigidity, and bradykinesia, which refers to slowness of movement when narrowly defined (Tatton and Lee, 1975; Logigian et al., 1991; Phillips et al., 1994). In addition, altered motor activities and anxiety-like behaviors are induced by the expression of mutant α-syn in mouse models of PD (Giasson et al., 2002; Unger et al., 2006; Nuber et al., 2008; Graham and Sidhu, 2010; Oaks et al., 2013).

Many of these clinical and pathological features have been reported in familial PD and mouse models of PD. However, the majority of PD cases are sporadic, and aging is the best predictor of the progression rate of sporadic PD and remains the most prominent risk factor for developing the disease (Post et al., 2007). We recently found that the earliest decline in mitochondrial function, as assessed by measuring

Abbreviations: PD, Parkinson's disease; CoQ, coenzyme Q; Q, oxidized coenzyme Q; QH_2, reduced coenzyme Q; OCR, oxygen consumption rate; α-syn, alpha-synuclein; VGluT1, vesicular glutamate transporter 1; SNc, substantia nigra pars compacta; LBs, Lewy bodies; TH, tyrosine hydroxylase.

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the mitochondrial oxygen consumption rate (OCR), during normal aging appeared in the brains of aged male mice at approximately 12 months of age (Takahashi and Takahashi, 2013). Mitochondrial dysfunction has been reported in PD (Hornykiewicz and Kish, 1987; Mizuno et al., 1989; Schapira et al., 1990; Parker et al., 2008); however, little is known regarding the behavioral and histological alterations that accompany reduced mitochondrial function in normal aging mice (Hirai et al., 2004). In this study, we examined whether the aging-associated decline in brain mitochondrial function is accompanied by an impairment of motor function and by Ser129-phosphorylated α-syn accumulation in specific regions of the brain. Furthermore, we examined whether a rescue of the reduced mitochondrial function by the exogenous administration of coenzyme Q (CoQ) to aged mice through drinking water (Takahashi and Takahashi, 2013) could restore the aging-associated changes in motor function and histological features if these changes occurred.

2. Materials and methods

2.1. Mice

C57BL/6NCr male mice were fed a standard chow ad libitum and were housed under pathogen-free barrier conditions with a 12 h-dark/light cycle. All protocols for animal use and experiments followed the Principles of Laboratory Animal Care (NIH publication No. 86-23, revised 1985), and the Animal Care Committee of the Tokyo Metropolitan Institute of Gerontology reviewed and approved all the study protocols. In certain experiments, 6- or 15-month-old mice (n = 6–9 per experiment) were fed a standard chow diet with drinking water containing 150 μM of water-soluble CoQ_{10} (122 mM CoQ_{10} emulsion with a mean diameter of 52 nm, Aqua Q10L10, Nissin Pharma Inc.) in a light-protected bottle for 7 days (Takahashi et al., 2012; Takahashi and Takahashi, 2013). Mice were sacrificed in the morning or underwent behavioral testing during the evening of the last treatment day.

2.2. Preparation of brain mitochondria

Brains were homogenized with a Dounce homogenizer (Wheaton) on ice in 20 volumes of a homogenizing buffer consisting of 0.25 M sucrose, 10 mM Tris–HCl, pH 7.4, 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride. Homogenates were centrifuged at 800 × *g* for 10 min at 4 °C, and the supernatant was centrifuged at 12,000 × *g* for 10 min at 4 °C to yield precipitates as a mitochondrial fraction. Aliquots of the mitochondrial fraction were used to measure protein content using a protein reagent kit (Thermo Fischer Scientific).

2.3. Measurement of the mitochondrial oxygen consumption rate

The oxygen consumption rate (OCR) of isolated mitochondria was determined at 37 °C using high-resolution respirometry (Oxygraph-2k, Oroboros Instruments) as previously described (Takahashi and Takahashi, 2013). DatLab software (Oroboros Instruments) was used for the data acquisition and analyses according to the manufacturer's protocols.

2.4. Measurement of mitochondrial CoQ content

The mitochondrial fraction was added to 4 volumes of 2-propanol and centrifuged at 12,000 rpm for 5 min at 20 °C. The supernatant was subjected to chromatography in a reverse-phase high-performance liquid chromatography (HPLC) system (LC-10Advp, Shimadzu), and CoQ content was detected using an electrochemical detector (ECD) (ESA Coulochem II, Thermo Fisher Scientific) as described previously (Takahashi and Takahashi, 2013).

2.5. Measurement of motor and behavioral activities

The pole test (PT) was first designed for use with mice to measure bradykinesia as a very sensitive assessment of nigrostriatal dysfunction (Ogawa et al., 1985, 1987; Matsuura et al., 1997; Hwang et al., 2005). For preoperative training, animals were placed facing upward on the top of a vertical wooden pole (13 × 450 mm) and were allowed to turn themselves downward and descend to the home cage filled with bedding material for 4 times. The next day after training, the animals received two test trials before performing the experimental test. The time required for the animals to orient themselves downward (T-turn) and the total time required to descend the length of the pole back to the floor of the cage (T-total) were measured, and the average of 5 trials was used as the final score.

The open field exploration test (OFT) has been proven to successfully measure emotional behavior in rats and mice (Christmas and Maxwell, 1970; Halcomb et al., 1975; Gentsch et al., 1987). The test provides a unique opportunity to systematically assess novel environmental exploration and general locomotive activity, and the test functions as an initial screen for anxiety-related behavior in rodents (Prut and Belzung, 2003).

Voluntary locomotor activity was assessed with the home cage activity test (HCT) (Ganea et al., 2007; Kitaoka et al., 2007; Kurokawa et al., 2011). An animal-movement analysis system (Shinfactory) consisting of a rectangular enclosure (20 × 30 cm) with a slide wall equipped with infrared sensors at 2-cm intervals was used, and each pair of photo sensors scanned animal movement at 0.5-s intervals. Food and water were provided ad libitum with a 12-h dark/light cycle. Total movements in each 10-min segment were automatically recorded for a weak, and the average of the total counts between the 4th and 6th day after a habituation period of three days was used as the final score.

2.6. Western blot analysis

The mitochondrial fraction in 0.25 M sucrose-containing homogenization buffer was directly lysed in Laemmli's sample buffer for 2 min for SDS-PAGE. In some experiments, whole brain lysates were treated prior to SDS-PAGE with alkaline phosphatase (FastAP, Thermo Scientific) at 0.3 units/μg protein for 1 h at 37 °C. After electrophoresis, the proteins were electrophoretically transferred onto a PVDF-FL membrane (Millipore), and the membrane was blocked with blocking buffer overnight at 4 °C. Proteins on the membrane were reacted with primary antibodies to Ser129-phosphorylated α-syn (1:1000, Abcam), α-syn (1:250, BD Biosciences), vesicular glutamate transporter 1 (VGluT1) (1:100, Invitrogen), tyrosine hydroxylase (TH) (1:1000, Millipore), and actin (1:200, Sigma), followed by IRDye 800CW-conjugated secondary antibody (1:10,000, Li-Cor Inc.). Protein bands were detected using an infrared imaging system (Odyssey, Li-Cor Inc.), and the band intensity was quantified using ImageJ software (National Institutes of Health).

2.7. Immunhistochemistry

Under anesthesia, the animals were transcardially perfused with PBS followed by 4% paraformaldehyde (PFA)/PBS. The brains were removed, and postfixed in 4% PFA/PBS for 48 h, dehydrated, and embedded in paraffin. The brains were serially sectioned at a thickness of 5 μm in the coronal plane. For immunostaining Ser129-phosphorylated α-syn and TH, the sections were treated with 0.3% hydrogen peroxide in methanol for 30 min, blocked in 10% normal horse serum for 30 min, and incubated with a primary antibody to Ser129-phosphorylated α-syn (1:20,000, pSyn#64, Wako) overnight at 4 °C or to TH (1:1000, Millipore) for 1 h at room temperature. After washing, the sections were incubated with the appropriate biotinylated secondary antibody for 30 min, followed by an avidin–biotin–peroxidase complex (Vector Laboratories) for 30 min. The sections were visualized using 3.3-diaminobenzidine