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Chronic mild stress damages mitochondrial ultrastructure and function in mouse brain

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ABSTRACT

Increasing evidence implicates mitochondrial failure as a crucial factor in the pathogenesis of mental disorders, such as depression. The aim of the present study was to investigate the effects of exposure to chronic mild stress (CMS), a paradigm developed in the late 1980s as an animal model of depression, on the mitochondrial function and mitochondrial ultrastructure in the mouse brain. The results showed that the CMS regime induced depressive-like symptoms in mice characterized by reduced sucrose preference and body weight. Moreover, CMS exposure was associated with a significant increase in immobility time in the tail suspension test. Exposure to the CMS paradigm inhibited mitochondrial respiration rates and dissipated mitochondrial membrane potential in hippocampus, cortex and hypothalamus of mice. In addition, we found a damaged mitochondrial ultrastructure in brains of mice exposed to CMS. These findings provide evidence for brain mitochondrial dysfunction and ultrastructural damage in a mouse model of depression. Moreover, these findings suggest that mitochondrial malfunction-induced oxidative injury could play a role in stress-related disorders such as depression.

Major depression is a serious and recurrent disorder manifested with symptoms at the psychological, behavioral and physiological levels. Depression affects 17–20% of the population of the world and may result in premature death, major social and economic consequences [10,24]. Among persons with major depression, 75–85% has recurrent episodes and 10–30% recovery incompletely and has persistent, residual depressive symptoms [14,30].

Increasing evidence implicates mitochondrial failure as a crucial factor in the pathogenesis of depressive disorder [26]. Mitochondria are the main site of energy production in eukaryotic cells. However, mitochondria are also a major source of reactive oxygen species (ROS) [25]. Under physiological conditions, ROS from mitochondrial respiratory chain could be reduced by intracellular antioxidant enzymes including superoxide dismutase, glutathione peroxidase and catalase as well as some antioxidant molecules such as glutathione and vitamin E [22]. Low levels of ROS are required for normal cell functions, i.e., cell signaling [15]. However, under pathological conditions, because of the elevated production, these antioxidants may fail to eliminate the large amount of ROS generated, leading to oxidative stress and cellular damage [16,32]. Thus, mitochondria are not only an important producer of ROS, but also a sensitive target for ROS [25]. Excessive generation of ROS could result in mitochondria dysfunction and activate mitochondria-dependent apoptotic pathway [7].

The brain is particularly vulnerable to reactive ROS production because it metabolizes 20% of total body oxygen and has a limited amount of antioxidant capacity [23]. Oxidative stress is well known to contribute to neuronal degeneration in brain during the aging process as well as in neurodegenerative diseases such as amyotrophic lateral sclerosis, Alzheimer's dementia and Parkinson's disease [23,31,32]. Recently, several studies have reported that ROS increased in the plasma of patients with major depression [19,26], suggesting oxidative stress-induced injury, especially mitochondrial dysfunction, is implicated in the pathogenesis of depression [26].

Numerous attempts have been made to set up animal models of depression or at least of some disease aspects. The chronic mild stress (CMS) model has been shown to induce lower consumption of sucrose (sweet food) postulated to reflect an hedonia (the loss of interest or pleasure) in animals, one of the two core symptoms required for diagnosis of a major depressive episode in humans [13,33]. The exposure of mice to CMS also induces changes in hypothalamic–pituitary–adrenal axis, body weight and adrenal glands all consistent with human depression [6,9]. Therefore, in the present study, we investigated the effects of CMS paradigm on mitochondrial function and mitochondrial ultrastructure in mouse

Abbreviations: ROS, reactive oxygen species; CMS, chronic mild stress; TST, tail suspension test; ST3, state 3 respiration; ST4, state 4 respiration; RCR, respiratory control ratio.

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brain to provide the direct evidence for mitochondrial dysfunction in depressive disorder.

C57 male mice (8–12 weeks, 25–32 g) were bred and maintained 4–5 mice per cage with a 12 h light/dark cycle at ambient temperature (22 °C) and relative humidity (55 ± 5%). All experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Guidelines for the Care and Use of Animals in Neuroscience Research by the Society for Neuroscience and approved by IACUC (Institutional Animal Care and Use Committee of Nanjing Medical University).

CMS was achieved as described previously [6]. Briefly, individually housed mice (n = 16–20 for each groups) were allowed to acclimate for 1 week and then were subjected to 6 weeks of stressors, which were mild and unpredictable in nature, duration, and frequency. Stressors included inversion of day/night light cycle, 45° tilted cage, restraint, overnight food and water deprivation, and pairing with another stressed animal. Sucrose preference was weekly monitored consistently throughout the course of experiments.

Before CMS procedure, animals were first trained to consume a sucrose solution. Mice were submitted to 3 days of continuous exposure to pipettes of water and of a 1% sucrose solution. After consumption stabilization, mice were divided in two groups, matched for their sucrose consumption baseline and their body weight baseline (n = 18). When perform sucrose preference test, mice were given the choice to drink from two bottles for 10 h; one contained a sucrose solution (1%) and the other contained only tap water. To prevent possible effects of side-preference in drinking behavior, the positions of the bottles in the cage were switched after 5 h. The animals were not deprived of food or water before the test. The consumption of tap water, sucrose solution and total intake of liquids was estimated simultaneously in the control and experimental groups by weighing the bottles. The mice whose sucrose consumption were high or low obviously than the others in the same groups were discarded in the later experiment. The preference for sucrose was measured as a percentage of the consumed sucrose solution relative to the total amount of liquid intake.

The TST is one of the most widely used models for assessing antidepressant-like activity in mice [27]. In this experiment, mice were individually suspended by the distal portion of their tails with adhesive tape for a period of 6 min (30 cm from the floor) in a visually isolated area. The time of immobility of the tail-suspended mice during the last 4 min was measured with a stopwatch.

Mitochondria were isolated by using a differential centrifugation method that retains mitochondrial structure and respiratory function [8]. Mitochondrial protein concentration was quantified according to Bradford using BSA as standard, and the mitochondria were diluted in isolation buffer to yield a fixed concentration according to the requirement of measurement.

The mitochondrial membrane potential ($\Delta\Psi_m$) was assessed with the fluorescent probe JC-1 (Molecular Probes). JC-1 is a cationic dye that exhibits potential-dependent accumulation in mitochondria, indicated by a fluorescence emission shift from green (~525 nm) to red (~590 nm). Mitochondria depolarization is indicated by a decrease in the red to green fluorescence intensity ratio. The green JC-1 signals were measured at Ex 485 nm (20 nm BW)/Em 535 nm (25 nm BW), the red signals at Ex 535 nm (25 nm BW)/Em 590 nm (20 nm BW). The ratio of red fluorescence signal (RFU at 590 nm) of JC-1 was recorded with a varioskan flash (Thermo scientific, USA). The initial protein concentration of isolated mitochondria for the staining procedure was 1 mg/ml. The detection was performed in 96 well plates.

Respiratory activities of mitochondrial preparations were measured by determining oxygen consumption using a Clark Electrode provided by Hansatech (King's Lynn, UK). The incubation medium was constantly stirred using an electromagnetic stirrer and bar flea. The oxygen consumption studies were conducted at 30 °C in respiration medium consisting of 25 mM sucrose, 75 mM mannitol, 95 mM KCl, 5 mM KH_2PO_4, 20 mM Tris–HCl, 1 mM EGTA, pH 7.4. The concentrations of substrates used were glutamate (5 mM) and malate (5 mM), which were added to the respiration media before the mitochondria were added. Approximately 0.5 mg of mitochondrial protein was preincubated in the oxygen electrode in a total volume of 1 ml with substrates for 5 min. State 3 respiration (ST3) was induced by the addition of adenosine diphosphate (ADP) (1 mM). Approximately 3 min later, ST3 was terminated and state 4 respiration (ST4) (resting) was detected. And the respiratory control ratio (RCR) was calculated from the ratio of the ST3/ST4 oxygen consumption rates.

After perfusion, cortex, hippocampus and hypothalamus of mice were microdissected from each mouse and were post-fixed in 2% Osmium tetraoxide at 0.1 M, pH 7.4 phosphate buffers at 48 °C for 1 h, and stained with uranyl acetate during 2 h. Later the sections were flat-embedded in Durcupan. Semi-thin (1 μm) sections were first stained with CFV and screened. Ultrathin sections of three regions were cut and placed on single-hole grids. After staining with uranyl acetate and lead citrate, the sections were examined by EM (Zeiss EM-9S).

All values are reported as means ± S.D. The significance of the difference between controls and samples treated with various drugs was determined by Student's t-test. Differences were considered significant at $P < 0.05$.

Stress-induced decrease in sucrose preference in rodents is regarded as an analog of anhedonia, a key symptom of depression. No significant difference of sucrose preference was found among unstressed mice. The CMS procedure significantly decreased the sucrose preference to 85 ± 7% compared to control mice (n = 18, $P < 0.05$) at the end of study (Fig. 1a).

TST—the behavioral models of antidepressant activity was to assess depression-related behaviors of mice. In the behavioral tests, immobility is interpreted as a "behavioral despair". The CMS procedure increased the immobility time in TST (137 ± 20 s), compared to control mice (114 ± 23 s, n = 18, $P < 0.05$) (Fig. 1b).

The effects of CMS on body weight are shown in Fig. 1c. At week 6, average body weight of control mice and CMS-treated mice were 27.6 ± 1.7 g and 21.6 ± 1.5 g, respectively. The body weight of stressed mice was reduced 2.6 g (11%) after 6-week-stress (n = 18, $P < 0.05$). The control mice gained weight over time, body weight of control mice increased 2.3 g (8%) at week 6 (n = 18, $P < 0.01$).

RCR is the major indices of mitochondrial function, which are determined based on measurements of oxygen utilization by mitochondria *in vitro*. Expose to CMS procedure reduced mitochondrial ST3 respiration rates in cortex, hippocampus and hypothalamus (n = 6, $P < 0.05$) (Fig. 2a) when the electron transport chain COX I substrates malate and glutamate were present, but failed to affect ST4 respiration rates (Fig. 2b). ST4 and ST3 respiration rates were used to calculate RCR. RCR of the mitochondria in the three brain regions were also remarkably decreased in CMS-treated mice (n = 6, $P < 0.01$) (Fig. 2c).

JC-1 can be used as an indicator of mitochondrial potential in a variety of cell types as well as in the isolated mitochondria. The most widely application of JC-1 is for detection of mitochondrial depolarization when occurring mitochondrial dysfunction or in the early stages of apoptosis. In the present study, we used the molecular probe JC-1 to detect the effects of CMS on $\Delta\Psi_m$. The results showed that CMS expose resulted in a loss of $\Delta\Psi_m$, as indicated by decreases in the red fluorescence signal (the value of RFU at 590 nm was shown in Table 1).

As shown in Fig. 3, swollen and vacuolated mitochondria were increased in the cortex, hypothalamus and hippocampus of mice exposed to CMS. The damaged mitochondria were markedly swollen, with broken or disrupted cristae or incomplete mem-