Research article

Potential involvement of the mitochondrial unfolded protein response in depressive-like symptoms in mice

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HIGHLIGHTS

• Chronic restraint stress induced depression-like behavior in mice.
• Chronic restraint stress decreased oxygen consumption rate in the forebrain.
• Chronic restraint stress increased levels of molecules associated with UPRmt.
• Levels of UPRmt-related molecules were correlated with depressive-like behavior.

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ABSTRACT

Many evidences strongly suggest that a mitochondrial deficit is implicated in major depression. A mitochondrial deficit leads to mitochondrial stress responses, including the mitochondrial unfolded protein response (UPRmt), which is associated with certain brain disorders such as spastic para- plegia and Parkinson’s disease. However, there is no evidence regarding the relationship between depressive disorder and UPRmt. Mice treated with chronic restraint stress showed significant depressive-like behaviors in the tail suspension and forced swim tests, decreased oxygen consumption rate, and increased levels of molecules associated with UPRmt such as Hspd9, Hspd1, Ubi5, Abcb10, and ClpP. All of the UPRmt-related molecules were significantly correlated with depressive-like behavior in the forced swim test. Thus, the present study is to reveal a relationship between the UPRmt and depressive disorder, suggesting that the UPRmt is a potential drug target for depressive disorders.

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1. Introduction

We hypothesized that mitochondria might be involved in the pathological condition underlying major depression for the following reasons. Mice with a mutated form of Polg1, in which the 3’-5’ exonucl ease activity was ablated and which caused mitochondrial DNA (mtDNA) deletions without affecting polymerase activity, show symptoms of bipolar disorder [1]. Human studies have shown that mitochondria from postmortem brain tissue of patients with major depression have lower levels of protein expression and enzymatic activity for ATP production compared with the mitochondria from healthy controls [2–4]. Interestingly, glutamatergic disturbances are known to contribute to the pathophysiology of mood disorders, and a carbon-13 magnetic resonance spectroscopy study revealed that the mitochondrial energy production of glutamatergic neurons was 26% lower in the depression group [5]. Additionally, patients with mitochondrial disorders, in which there are inherent functional deficits in mitochondrial energy production, have an increased risk for developing major depression [6,7]. Moreover, CoQ10 and l-carnitine, are the components of medicines that are commonly used to treat mitochondrial dysfunction, which have been shown to improve the symptoms of major depression [8]. These findings strongly implicate a mitochondrial deficit in major depression.

Mitochondrial deficit causes a decrease in oxygen consumption, which is observed in many rodent models of depression. The partial pressure of oxygen was found to be reduced in the cortex and striatum in two different rodent models of depression: chronic corticosterone treatment or unpredictable mild stress in rats [9]. In addition, chronic mild stress decreases the oxygen consumption of mitochondria in the cortex, hippocampus, and hypothalamus of mice [10]. The number of damaged mitochondria, which were markedly swollen, with broken or disrupted cristae, or incomplete
membranes, was also increased. Thus, the reduction of oxygen consumption is observed in depression, but it is unknown how this reduction occurs.

Mitochondrial unfolded protein response (UPRmt) is a stress response that activates the transcription of nuclear-encoded mitochondrial chaperones to promote protein homeostasis [11]. It is accompanied by a change in oxygen consumption rate [12]. During the UPRmt the AAA+-protease ClpP degrades unfolded proteins to peptides that might be transported across the inner membrane through the ATP-binding cassette transporter, Abcb10, which is most likely the mouse homologue of haf-1 from C. elegans. Ubl-5 binds to the promoters of the mitochondrial chaperones, Hspa9 and Hspd1, to promote transcriptional activation. The upregulation of mitochondrial chaperones contributes to relieving stress and re-establishing homeostasis [11]. The UPRmt is known to be associated with some brain disorders such as spastic paraplegia and Parkinson's disease. Spastic paraplegia is a neurodegenerative disorder caused by 13 mutations including in the mitochondrial chaperone, Hspd1 [13]. In addition, Parkinson's disease-associated variants in Hspa9 gene cause an impairment of mitochondrial function and dynamics [14]. However, to the best of our knowledge, there is no current evidence regarding the relationship between depressive disorder and UPRmt.

In present study, we attempted to clarify the relationship between these mitochondrial deficits and depressive-like behavior by focusing on oxygen consumption and the UPRmt in a mouse model of depression.

2. Materials and methods

2.1. Animals

Six-week-old male C57BL/6j mice were obtained from CLEA Japan Inc. (Tokyo, Japan) and habituated for 1 week before starting the experiments. Mice were maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.) at a constant temperature of 22 °C ± 1 °C with ad libitum access to food and water throughout the experiment, unless specified otherwise. All experiments were performed in the light cycle from 9:00 a.m. to 12:00 p.m. approved by the Institutional Animal Care and Use Committee of Kagoshima University (ID: MD12058), and performed in accordance with the guidelines for the care and use of animals published by the Pharmacological Society of Japan and the Institutional Animal care and Use Committee of Kagoshima University.

2.2. Chronic restraint stress

The restraint treatment used in this study was performed as previously described with minor modifications [15]. Mice were placed alone in a well-ventilated acrylic tube (internal diameter, 3 cm; internal length, 8 cm) and restrained for 2 h from 09:00 a.m. to 11:00 a.m. daily for 14 days. Fig. 1A illustrates the schedule for restraint stress, assays, and behavioral tests. On day 1 after the final session of restraint stress, forebrain samples were obtained from a group of mice for the oxygen consumption measurements and reverse transcription–quantitative polymerase chain reactions (RT-qPCRs). The tail suspension test and forced swim test were then performed on the 8th and 10–11th days after the final session of restraint stress. Forebrain samples were then obtained from mice on day 14 for the oxygen consumption measurements, RT-qPCR, and malondialdehyde (MDA) assays.

2.3. Tail suspension test

The tail suspension test used in this study was performed as previously described with minor modifications [16]. Each mouse was suspended by fixing its tail 2 cm in the center of the upper surface using a tail hanger and non-irritating adhesive tape. The duration of immobility in 1-min intervals for 6 min was automatically evaluated using the activity measuring and recording system, Supermex-CompACT AMS instrument (Muromachi Kikai Co., Ltd., Tokyo, Japan). This instrument uses an infrared sensor that was placed behind the mouse body to detect any movement.

2.4. Forced swimming test

The forced swimming test used in this study was performed as previously described with minor modifications [17]. A glass cylinder apparatus (height = 25 cm, diameter = 19 cm) containing 15 cm of water at 21–23 °C was used. On the first day, mice were placed individually in the water and allowed to swim for 15 min. The next day, mice were placed again in the water to observe immobility for 6 min. The duration of immobility was evaluated at 1-min intervals, for 6 min, using the Supermex-CompACT AMS instrument.

2.5. Brain mitochondria isolation and measurement of oxygen consumption

Brain mitochondria isolation and the measurement of oxygen consumption were performed as previously described [18]. Briefly, mice (6 mice for one day or 9–12 mice for 14 days after final restraint stress) were sacrificed, and the forebrain was weighed, transferred into 9× volume of the ice-cold MSE solution (225 mM mannitol, 75 mM sucrose, 1 mM EGTA, 5 mM HEPES, 1 mg/ml bovine serum albumin, pH 7.4) containing 0.05% nagarse, and homogenized at 600 rpm using a glass–Teflon homogenizer. Thereafter, the homogenate was centrifuged at 2000 × g for 4 min; the supernatant was collected and subsequently centrifuged at 12,000 × g for 9 min. To permeabilize the synaptosomes, the resulting pellet was dissolved in 1-ml ice-cold MSE solution containing 0.02% digitonin. The solution was transferred to a small glass homogenizer (small clearance) and homogenized 8 strokes. The homogenized suspension was centrifuged at 12,000 × g for 11 min, followed by resuspension of the pellet in MTP solution (10 mM KH2PO4, 60 mM KCl, 60 mM Tris–HCl, 110 mM mannitol, 0.5 mM EGTA, 10 mM glutamate, 5 mM malate, 3.3 mM MgCl2, pH 7.4) as mitochondrial fraction. The protein concentration of mitochondrial fraction was measured using the Bradford method with a Bio-Rad Protein assay kit (Bio Rad Tokyo, Japan).

To measure basal dissolved oxygen, 100 μL of air-equilibrated MTP solution at 30 °C was added to the Mitocell precision respiratory system (Strathkelvin Instrument, North Lanarkshire, UK), equipped with an oxygen electrode, for 1 min. Isolated forebrain mitochondria, up to 100 μg protein, was added to the chamber to observe steady-state respiration for 2 min, and then 250 μM adenosine diphosphate (ADP) was added to observe state 3 and 4 respiration for 2 min.

2.6. Total RNA isolation and RT-qPCR

Mice (9 mice for each of CTRL and Restraint group) were sacrificed, and forebrain samples were treated with TRIzol® LS (Life Technologies, Tokyo, Japan) and total RNA was isolated according to manufacturer’s protocol. The obtained total RNA was treated with DNase (Promega KK, Tokyo, Japan) and subsequent phenol/chloroform/ethanol purification. Amount of total RNA was measured using a Nano-drop 1000 (Thermo Scientific, Kanagawa, Japan), and reverse-transcribed using high capacity cDNA RT kit (Life Technologies, Tokyo, Japan). qPCR was performed with equal amounts of cDNA and appropriate target primers to assess the levels of gene expression with SYBR Premix Ex Taq II (Takara bio Inc., Shiga, Japan) and a Thermal Cycler Dice (Takara bio Inc., Shiga, Japan).
Japan), according to the manufacturer protocol. The primers used are listed in Supplementary Table.

2.7. MDA assay

The MDA assay was performed as previously described with minor modifications [19]. Briefly, forebrain lysates were treated with trichloroacetic acid in order to precipitate the proteins, and the supernatant was reacted with 2-thiobarbituric acid at 100 °C for 10 min. Measurement of the absorbance of the reacted product at 532 nm was performed with an Infinite 200 microplate reader (Tecan Japan Co., Ltd., Kanagawa, Japan)

2.8. Statistical analysis

Values are shown as mean ± standard error (SE). Statistical significance was examined using Student’s t-tests to compare means or Spearman’s rank test to analyze any correlation between groups. All analyses were performed with Prism 5 software (Graph Pad-San Diego, CA, USA).

3. Results

The chronic restraint stress model used in the present study was based on a previous report [18], in which depressive-like behavior persisted for 12 weeks following chronic restraint stress and was recovered with imipramine treatment for 2 weeks [15,20]. Consistent with this finding, mice showed significant depressive-like behaviors in both the tail suspension test (Fig. 1B and C) and forced swim test (Fig. 1D and E) following daily chronic restraint stress for 2 weeks.

Chronic stress in mice decreases mitochondrial oxygen consumption in mitochondria obtained from the cortex, hippocampus, and hypothalamus [10], suggesting that chronic stress affects mitochondria throughout the forebrain. Therefore, we investigated the mitochondrial oxygen consumption of the whole forebrain. Following the addition of isolated mitochondria from the forebrain into the chamber, dissolved oxygen decreased in a time dependent manner (steady state). The oxygen consumption rate was facilitated by adding ADP (state 3), and subsequently consumption decreased after the deprivation of ADP in the chamber (state 4). The slopes of the linear curve for respirations in steady state, state 3, and state 4 were quantified as the corresponding oxygen consumption rates (Fig. 2A). Although, the oxygen consumption rate did not significantly changed between steady state and state 4, it was decreased in state 3 one day after chronic restraint stress (Fig. 2B), and this decrease persisted up to 2 weeks after the stress (Fig. 2C).

The UPRmt has been shown to accompany changes in the oxygen consumption rate [12]. Therefore, the expression of UPRmt-related genes, such as Hspa9, Hspd1, Ubis, Abcb10, and ClpP, were...
assessed by qPCR in naïve and depressed mice. Although the levels of expression of all these genes were unchanged one day after chronic restraint stress (Fig. 3A), they were upregulated 14 days after the final stress (Fig. 3B). The relationship between depressive-like behavior in the forced swim test and the expression of the UPRmt genes was analyzed with the Spearman’s rank correlation test, which showed that the levels of expression of all of these genes were positively correlated with the depressive-like behavior (Fig. 3C–G). In addition, depressive-like behavior on the tail suspension test was positively correlated with the expression levels of these proteins (data not shown). In agreement with a previous report that reactive oxygen species (ROS) activate UPRmt [11], the levels of MDA, which is the end product of lipid peroxidation, were increased in the mouse model of depression (Fig. 3H).

4. Discussion

The significance of present study is that this is the first demonstration of a correlation between the UPRmt and depressive-like behaviors in mice. There was a significant increase in the expression of genes associated with the UPRmt which might be a signature of mitochondrial unfolded protein stress in the depressed mouse. However, it is unknown which the UPRmt or unfolded protein stress itself is correlated with depressive disorder. Mutations in HSPA9 and HSPD1 cause spas tic paraplegia and Parkinson’s disease, respectively; suggested that inherited dysfunction of mitochondrial chaperones might lead to mitochondrial unfolded protein stress and result in brain disorders [13,14]. Meanwhile, another study showed that Hsp60-(p.V98I) mutation associated with spas tic paraplegia did not globally increase protein aggregation in a bacterial model system [21], which suggests the possibility that unfolded protein stress was not mediated in these brain disorders. Thus, it is difficult to conclude that mitochondrial unfolded protein stress, which is caused by aggregated protein, leads to such brain disorders. If the unfolded or aggregated proteins in mitochondria were observed in brain of patients with depressive disorder or animal models of depression, we could conclude mitochondrial unfolded protein stress might be involved in depressive disorder.

The levels of UPRmt related molecules in mice with chronic restraint stress were higher than that in naïve mice, suggesting that depression model mouse exhibited mitochondrial dysfunction. Although, many papers also reported that mitochondrial dysfunction is observed in patients with major depression [3–5], as well as rodent models of depression [9,10], how this mitochondrial deficit occurs in depressive disorders remains unclear. In our result, MDA was increased in the brain from depressive model mice, and previous reports showed that chronic restraint stress increases hippocampal ROS via NADPH oxidase in mice [15]. In addition, chronic unpredictable stress or chronic injections of corticosterone increased ROS, and decrease of the expression of mitochondrial superoxide dismutase 2 and glutathione, ROS scavengers, in the prefrontal cortex of rats [9]. Furthermore, another ROS scavenger ascorbic acid can reduce depressive-like behavior mediated by chronic unpredictable stress [22]. One human study reported a significant relationship between the concentration of serum reactive oxygen metabolites and Beck Depression Inventory scores [23]. Thus, ROS might be involved in depressive disorder. Moreover, mtDNA is a critical cellular target for ROS, because the mtDNA damage is more extensive and persists longer than nuclear DNA damage following oxidative stress [24]. These findings suggest that ROS production mediated by excess corticosterone, might lead to a mitochondrial deficit in the progression of depressive disorder.

The question of how a mitochondrial deficit can lead to depression remains. The underlying mechanism might be explained by disturbances in the glutamatergic system. Depressive behavior has been shown to be accompanied by an imbalance in glutamate transmission [25], which is modulated by the neuroinflammatory pathway, and this imbalance is accompanied by ROS production [26]. Interestingly, the oxidative energy production in
mitochondria is selectively reduced in glutamatergic neurons from individuals with major depression [5]. Thus, disturbances in the glutamatergic system by the mitochondrial deficit might result in depression symptoms.

The UPRmt-related genes were upregulated during the 14 days after the final restraint stress. Interestingly, it has been shown that the onset of anxiety behavior takes 10 days after restraint stress, and that spine density increases in accordance with the anxiety behavior [27]. This report suggests a time-lapse between the occurrence of the stressful incident and the induction of behavioral changes and synaptic plasticity. Thus, time might be needed for the induction of UPRmt as well, and UPRmt might occur in association with synaptic plasticity. However, future experiments are required to understand the significance of this time-lapse between the incidence of stress and the induction of UPRmt on the pathophysiology of depression.

5. Conclusions

In conclusion, we have shown that the UPRmt may be related to the pathophysiology of major depression and a potential drug target for depressive disorder.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neulet.2015.01.006.

References
