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Research article

Rapamycin relieves anxious emotion and synaptic plasticity deficits induced by hindlimb unloading in mice

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ABSTRACT

This study examined whether increasing autophagy could improve cognitive deficits in hindlimb unloaded (HU) mice, which was used as an animal model of synaptic plasticity impairment. Male C57BL/6 mice were randomly divided into three groups: control, HU and HU + rapamycin groups. Hindlimb unloading treatment was used to establish the animal model for 2 weeks. Rapamycin was intraperitoneally injected at a dose of 0.5 mg/kg/day along with hindlimb unloading procedure. The open field test and the elevated plus maze test showed that rapamycin considerably prevented the level of anxiety and increased exploratory behaviour in HU mice. Afterwards, long-term potentiation (LTP) recorded in the hippocampal dentate gyrus (DG) region was effectively reduced by rapamycin, which was significantly inhibited by HU procedure. In addition, rapamycin further increased the autophagy level, which was already elevated in HU mice. Meanwhile, the expression of NMDA receptor 2A and 2 B was modified by rapamycin in HU mice. Moreover, rapamycin noticeably increased the total superoxide dismutase (T-SOD) activity and reduced the malondialdehyde (MDA) as well as the level of carbonylated proteins in HU mice's hippocampus. The results show that increasing autophagy may pacificate the anxious emotion, and partly alleviate the hippocampal synaptic plasticity deficits.

1. Introduction

There are numerous adverse factors in space, and microgravity is one of the major causes of the impairment of neurological capabilities, such as performance, locomotion, memory, learning and coordination, leading to potential risks in space missions [10]. The hindlimb unloading rodent model has been widely used for researcher as an animal model of simulating microgravity since it was accepted by National Aeronautics and Space Administration (NASA) during mid-1970s [12]. One of our previous studies showed that HU mice exhibited depression-like and anxiety-like behaviours [18]. The rat's learning and memory ability was significantly impaired by HU procedure [4,22]. However, the underlying mechanisms of emotion and cognition deficits, induced by HU, still need to explore.

Autophagy is an evolutionary conserved degradative mechanism involved in the recycling of cytoplasmic components in eukaryotic cells [7]. Normally, the autophagy pathway is inhibited by the mammalian target of rapamycin (mTOR) pathway. mTOR inhibits the activity of the ULK1 complex, which is essential for autophagosome biogenesis [9]. Therefore, as an inhibitor of the mTOR, rapamycin could activate autophagy pathway. Lots of studies showed that constitutive autophagy was necessary for neuronal survival [14]. The activation of autophagy could attenuate neuron injurious and enhance anxiolytic and exploratory behaviours in ischemic rats [16]. Furthermore, the activation of autophagy could alleviate both cognition deficits and synaptic plasticity impairments in melamine-treated rats [6]. Oxidative stress plays an important role in the occurrence and development of neurodegenerative diseases [3]. Previous studies showed that the reactive oxygen species (ROS) level was increased in the serum of two weeks HU rats [20] and in the hippocampus of three weeks HU rats [21]. Importantly, oxidative damage could induce autophagy, while autophagy can also selectively remove oxidatively damaged proteins and organelles such as mitochondria [5]. It is generally believed that ROS are mainly produced by intracellular mitochondria respiratory chain. Logically, the interaction between ROS and autophagy may play a vital role in HU animals.

Previously, we found that HU procedure could induce anxiety-like behaviours in mice and significantly change the pattern of neural oscillations in mice's hippocampus [18], but the underlying mechanism is still unclear to some extent. In this study, we examined whether or not autophagy can play an important role in improving cognitive

Abbreviations: HU, hindlimb unloaded; T-SOD, total superoxide dismutase; MDA, malondialdehyde; PP, perforant pathway; Rap, rapamycin; OFT, open field test; EPM, elevated plus maze test; Bnip3, Bcl-2/E1B-19 kDa interacting protein 3

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impairments induced by HU procedure via reducing ROS. Accordingly, a mouse model of HU was established and rapamycin was used to regulate autophagy. Afterwards, behaviour tests were performed and LTP from the hippocampal perforant pathway (PP) to dentate gyrus (DG) area was recorded. Since the activation of synaptic N-methyl-D-aspartate receptors (NMDAR) is important in LTP induction [13], the expression of NR2A and NR2B was measured. In addition, calcium-calmodulin-dependent kinase II (CaMKII), which is necessary and sufficient for LTP [13], was measured as well. In order to explore the potential mechanism, specific markers of autophagy were measured by Western blot assay. Moreover, to evaluate whether upregulating autophagy could improve the damage of cognitive functions and synaptic plasticity via decreased ROS levels, both MDA/T-SOD and the level of carbonylated proteins were measured to evaluate intracellular ROS levels.

2. Materials and methods

All experiments were done on the basis of protocols approved by the Ethical Commission at Nankai University and met the practices guidelines in the NIH Guide for the Care and Use of Laboratory Animals. The body weight profile was showed in Fig. S1. The details can be seen in the Supplementary materials.

3. Results

3.1. The performances of mice in open field test and elevated plus maze test

The results of the OFT and EPM were showed in Fig. 1 (OFT: Fig. 1A–C, and 1F; EPM: Fig. 1D–F). Post hoc test showed that the time spent in the central area (Fig. 1A, $p < 0.05$), central area entries (Fig. 1B, $p < 0.05$) and vertical activity score (Fig. 1C, $p < 0.001$) were much less in the HU group than that in the Con group, while there was no statistical difference of these parameters between the Con group and the HU + Rap group (Fig. 1A–C, $p > 0.05$ respectively) in OFT.

The EPM test showed that the time spent in open arms (Fig. 1D, $p < 0.05$) and the percentage of open arms entries (Fig. 1E, $p < 0.001$) were markedly decreased in the HU group than that in the Con group. Interestingly, rapamycin significantly increased the time spent in open arms (Fig. 1D, $p < 0.05$) and the percentage of open arms entries (Fig. 1E, $p < 0.001$) in the HU + Rap group compared to that in the HU group.

To further distinguish the effects of HU on motor ability and cognitive functions, the immobile time of mice during both the OFT and the EPM tests was evaluated. There were no statistical differences of the immobile time between the groups (Fig. 1F, $p > 0.05$). The data suggested that HU treatment induced anxious emotion in the mice, however rapamycin could reduce anxiety-like behaviour.

3.2. LTP from PP to DG

In the LTP test, the slope of basal field excitatory postsynaptic potentials (fEPSPs) was considerably increased after theta burst stimulation and then stabilized to a level above the baseline period in all the three groups (Fig. 2A). The inset in Fig. 2A presents an instance of fEPSPs at the baseline and LTP of a mouse in the Con group. The last 10-min recordings were analyzed to be the group data. Bonferroni post hoc test showed that the mean fEPSPs slopes were significantly smaller in the HU group than that in the Con group (Fig. 2B, $p < 0.001$). However, they were dramatically increased in the HU + Rap group compared to those in the HU group (Fig. 2B, $p < 0.001$).

3.3. Measurements of NR2A, NR2B and CaMKII expression

To examine the effects of rapamycin on the expression of NR2A, NR2B and CaMKII in hindlimb unloading-treated mice, three prominent bands at about 180 kDa, 163 kDa and 45 kDa were detected by NR2B, NR2A and CaMkII antibodies, respectively (Fig. 3A–D).The results

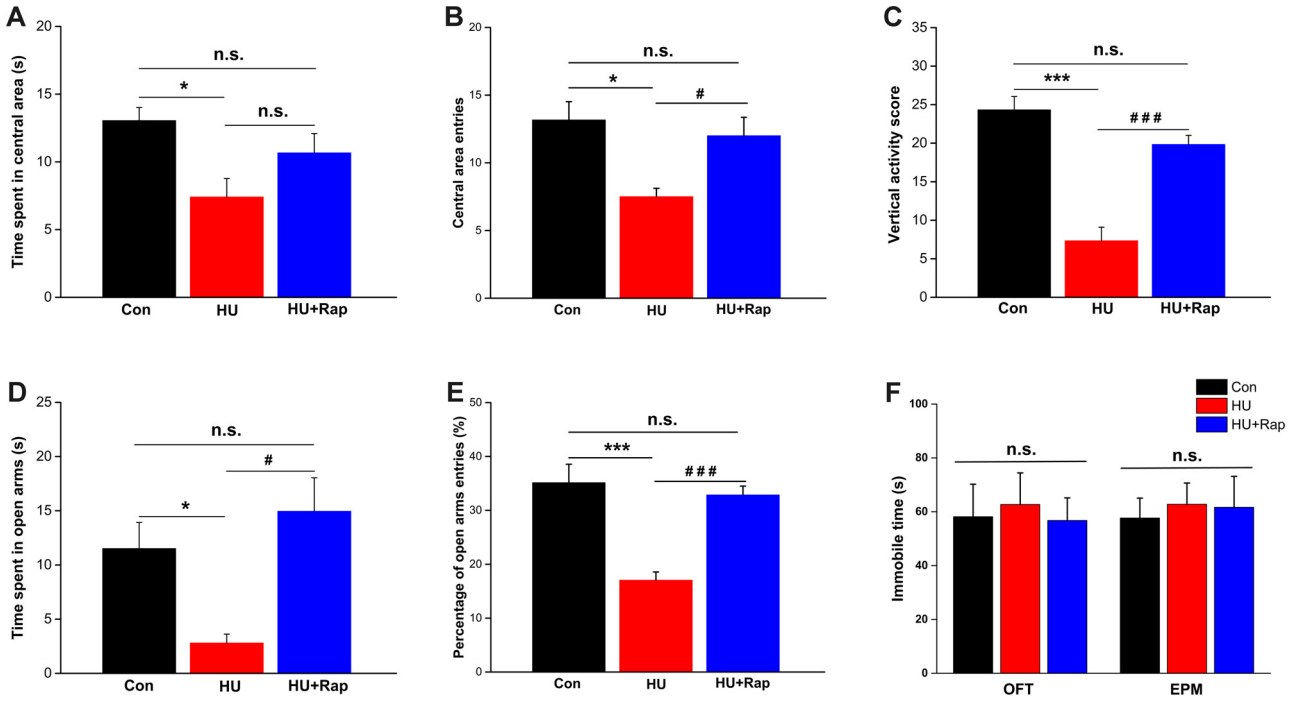

Fig. 1. The performances of mice in both behavioral tests (OFT and EPM).
The effects of rapamycin on the time spent in the central area (A), central area entries (B), and vertical activity (C) in the open field test after two weeks HU treatment. The effects of rapamycin on the time spent in the open arms (D), and percentage of open arms entries (E) in the elevated plus maze test. The immobile time in OFT and EPM (F).
Data are presented as mean ± SEM. * $p < 0.05$, *** $p < 0.001$ v.s. Con group; # $p < 0.05$, ### $p < 0.001$ v.s. HU group; n.s. $p > 0.05$. n = 6 in each group.