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Repeated restraint stress increases seizure susceptibility by activation of hippocampal endoplasmic reticulum stress

Xinjian Zhu [a, *], Jingde Dong [b], Zhengrong Xia [c], Aifeng Zhang [d], Jie Chao [e], Honghong Yao [a]

[a] Department of Pharmacology, Medical School of Southeast University, Nanjing, China
[b] Department of Geriatric Neurology, Nanjing Brain Hospital Affiliated to Nanjing Medical University, Nanjing, China
[c] Analysis and Test Center of Nanjing Medical University, Nanjing, China
[d] Department of Pathology, Medical School of Southeast University, Nanjing, China
[e] Department of Physiology, Medical School of Southeast University, Nanjing, China

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ABSTRACT

A growing body of evidence suggests that stress triggers a variety of pathophysiological responses. Recent studies show that stress produces enduring effects on structure and function of hippocampus, which is one of the most important structures involved in epilepsy. In the present study, we determined the effect of repeated restraint stress exposure on the susceptibility of pentylenetetrazole (PTZ)-induced seizures and the possible mechanisms involved using a rodent model. Our results show that mice subjected to repeated restraint stress exhibited shorter latency to PTZ-induced tonic-clonic seizures and higher seizure severity, suggesting chronic restraint stress increases seizure susceptibility. Following repeated restraint stress, we observed an increased level of endoplasmic reticulum (ER) stress as well as oxidative stress in the hippocampus. Moreover, our results show that chronic restraint stress exposure causes neuron loss in the hippocampus. Inhibition of ER stress with chemical chaperone, tauroursodeoxycholic acid (TUDCA), however, protects against chronic restraint stress-induced neuron loss, suggesting repeated restraint stress-induced neuronal degeneration is dependent on ER stress activation. On the other hand, inhibition of ER stress with TUDCA suppresses restraint stress-induced seizure susceptibility. Taken together, these results indicate that repeated restraint stress increases seizure susceptibility by activation of hippocampal ER stress and ER stress mediated oxidative stress and neurodegeneration. Thus, attenuating ER stress may serve as a potential therapeutic strategy targeted to block stress-induced seizure activities.

1. Introduction

Prolonged stress exposure induces alterations in hippocampal network and neuronal excitability. Several lines of evidence suggest that chronic stress can affect hippocampal synaptic plasticity (Abush and Akirav, 2013; Joels et al., 2007), dendrite spine density (Radley et al., 2013; Rico et al., 2015) and neurogenesis (Snyder et al., 2011). Hippocampus is often considered as the focus of epileptic seizures, and maladaptive changes of hippocampus induced by stress exposure may increase the vulnerability to seizure. In clinic, patients with epilepsy commonly report that stress is a precipitating factor of seizures (Fisher et al., 2000; Maguire and Salpekar, 2013; Nakken et al., 2005). In animal studies, it was demonstrated that repeated stress exposure increases seizure susceptibility (Chadda and Devaud, 2004; Jones et al., 2013). Despite a large amount of experimental evidence support that chronic stress induces seizure susceptibility, it is still unclear how chronic stress contributes to the development of epilepsy or precipitation of seizure.

The endoplasmic reticulum (ER) is responsible for generating and folding secreted and membrane proteins. Disruption of the protein folding or accumulation of misfolded proteins in the ER induces a pathological state known as ER stress. ER stress triggers an adaptive response called the unfolded protein response (UPR), which is a signal transduction pathway responsible for restoring

* Corresponding author. Department of Pharmacology, Medical School of Southeast University, Dingjiaqiao 87th, Nanjing, 210009, China.
E-mail address: xinjianzhu@seu.edu.cn (X. Zhu).

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protein folding and degradation of misfolded proteins (Lindholm et al., 2006; Xu et al., 2005). However, under chronic ER stress, the UPR will elicit the signals leading to apoptosis (Tabas and Ron, 2011; Urra et al., 2013) and cell death (Walter and Ron, 2011). ER stress is activated and regulated by a variety of factors. Recent studies report that chronic stress can disrupt protein folding process, and consequently leads to ER stress in the central nervous system including cortex, hippocampus and amygdala (Huang et al., 2015; Zhang et al., 2014; Zhao et al., 2013), suggesting a role of ER stress in chronic stress-related neuropathogenesis. ER stress is now implicated in the pathogenesis of several neurological disorders including epilepsy (Bouman et al., 2011; Brennan et al., 2013; Han et al., 2015; Hoozemans et al., 2009; O'Connor et al., 2008; Torres-Peraza et al., 2013; Yamamoto et al., 2006; Zhao et al., 2016). However, the precise molecular mechanism by which ER stress contributes to these neurological diseases remains largely elusive, particularly the mechanisms underlying the effects of ER stress on seizure and epilepsy. Here in this study, we sought to determine whether chronic stress induces seizure susceptibility and to further explore the role of ER stress on chronic stress-induced seizure activity.

2. Materials and methods

2.1. Animals

Male C57/BL6 mice (4 weeks old; weighing 19 ± 2 g at the beginning of the experiments) were obtained from the comparative medicine center of Yangzhou University (Yangzhou, China). The animals were housed in plastic cages and kept in a regulated environment (22 ± 1 °C) with an artificial 12 h light/dark cycle (lighted from 7:00 a.m. to 7:00 p.m.). Food and tap water were available *ad libitum*. Procedures for PTZ induced-seizures and all subsequent experiments were approved by the Animal Care and Use Committee of Medical School of Southeast University. All efforts were made to minimize animal suffering and discomfort and to reduce the number of animals used.

2.2. Drugs

Pentylenetetrazole (PTZ) and tauroursodeoxycholic acid (TUDCA) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Repeated restraint stress procedure

Animals were maintained under standard laboratory conditions for at least 7 days before starting the stress protocol. For repeated restraint stress, we used a modified version of the restraint protocol described by Mozhui et al., (2010). Briefly, mice were placed in air-accessible cylinders for 2 h daily (14:00 p.m. to 16:00 p.m.) for 3, 7 and 14 consecutive days. Immediately following the final restraint stress, mice were subjected to seizure susceptibility assay or sacrificed to obtain brain tissue for further experiment. To investigate the role of ER stress on oxidative stress and seizure susceptibility, mice were treated with an ER stress inhibitor TUDCA during the 14 days restraint stress procedure. Mice were subjected to seizure susceptibility assay or sacrificed to obtain brain tissue for further experiment as described above.

2.4. PTZ-induced seizures

PTZ was administered intraperitoneally to induce acute seizure at a single dose of 60 mg/kg according to the previous studies (Abdallah, 2010; Mizoguchi et al., 2011; Uma Devi et al., 2006). Mice were then subjected to continuous video monitoring for 30 mins following PTZ administration to observe seizure events. The seizure intensity was scored as follows Stage 0, no response; Stage 1, ear and facial twitching; Stage 2, convulsive twitching axially through the body; Stage 3, myoclonic jerks and rearing; Stage 4, turning over onto the side, wild running, and wild jumping; Stage 5, generalized tonic-clonic seizures; and Stage 6, death (Becker et al., 1995; Mizoguchi et al., 2011; Schroder et al., 1993). Latency to the onset of tonic-clonic seizures, the seizure scores in 5-min blocks during 30 min, and the number of mortality were measured.

2.5. Intracerebroventricular (i.c.v.) injection

For i.c.v. injection, a guide cannula (33-gauge, Plastics One Inc., Roanoke, VA, USA) was implanted in the right lateral ventricle. Briefly, mice were anesthetized and positioned in a stereotaxic instrument with a mouse adapter (David Kopf Instruments, Tujunga, CA, USA). The stereotaxic coordinates for implantation of guide cannula into right lateral ventricle were according to the mouse brain atlas (AP = 0.5 mm relative to bregama; ML = 0.8 mm; DV = −2.5 mm from the skull surface). The guide cannula was then affixed with dental cement and mice were allowed to recover for at least 7 days. After recovery, the patency of the cannula was tested by injection of 250 ng of angiotensinIIin 1.0 μl PBS, since angiotensinIIinduces drinking response by stimulating preoptic structure (Olivadoti and Opp, 2008; Skott, 2003). Mice with positive drinking response were selected in the subsequent experiment. Injections were performed using a Hamilton syringe attached to the guide cannula. Vehicle (PBS), and vehicle containing TUDCA (1 μg) were injected in control and repeated restraint stressed mice during the 14 days restraint stress procedure every other day for 7 dose (Purkayastha et al., 2011; Young et al., 2012). All injections were 1 μl and the injections were carried out over 60 s and the syringe was left in place for additional 2 min to minimize backflow after each injection. Mice were then subjected to seizure susceptibility assay or sacrificed to obtain brain tissue for further experiment as described above.

2.6. Brain tissue processing

For western blot experiment, the hippocampus was dissected from control and restraint stressed mice. Dissected hippocampal tissues were then snap-frozen and stored at −80 °C until use. For immunocytochemistry experiment, the mice were euthanized by an intraperitoneal injection of an overdose of urethane and were transcardially perfused with 100 mL of saline (0.9% w/v NaCl), followed by 50 mL of 4% paraformaldehyde in 0.05 M sodium phosphate (pH = 7.4, containing 0.8% NaCl). The mouse brains were removed and post-fixed overnight in 4% paraformaldehyde, then were cryoprotected in 30% sucrose in 1xPBS for 72 h. Serial coronal hippocampal sections with a thickness of 25 μm were cut using a cryostat (Leica Microsystems, Wetzlar, Germany) and every sixth section throughout the hippocampus were collected in 1× PBS as free-floating sections and were stored at 4 °C for future immunocytochemistry studies as described previously (Zhu et al., 2016). For the transmission electron microscopy (TEM) analyses, mice were deeply anesthetized and transcardially perfused with 0.1 M phosphate buffer (PBS, pH = 7.4), followed by 4% PFA and 2% glutaraldehyde. The CA3 subfield of the hippocampus was then removed and processed for electron microscopy.

2.7. Immunocytochemistry and cell counting

The immunocytochemistry studies were performed on free-floating sections as described previously (Zhu et al., 2016). Briefly, the sections were heated (65 °C for 50 min) in antigen unmasking