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Endoplasmic reticulum stress is involved in restraint stress-induced hippocampal apoptosis and cognitive impairments in rats

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HIGHLIGHTS

- RS induces hippocampal apoptosis and cognitive impairments.
- RS triggers the UPR in hippocampus.
- The ERS-induced apoptotic pathway is activated in hippocampus of RS rats.
- Inhibition of ERS alleviates the cognitive impairments induced by RS.

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ABSTRACT

Long-term exposure to stressful stimuli can reduce hippocampal volume and cause cognitive impairments, but the underlying mechanisms are not well understood. Endoplasmic reticulum stress (ERS) is considered an early or initial response of cells under stress and linked to neuronal death in various neurodegenerative diseases. The present study investigated the involvement of ERS in restraint stress (RS)-induced hippocampal apoptosis and cognitive impairments. Using the rat RS model for 21 consecutive days, we found that the hippocampal apoptotic rate was significantly up-regulated as compared with unstressed controls, and salubrinal (ERS inhibitor) pretreatment effectively reduced the increase. As the marker of ERS, the 78-kDa glucose-regulated protein (GRP78) and the target molecule of the unfolded protein response (UPR), the splice variant of X-box binding protein 1 (sXBP-1) were also markedly increased in RS rats. Furthermore, in the three possible signaling pathways of ERS-induced apoptosis, the protein and mRNA levels of C/EBP homologous protein (CHOP) were significantly up-regulated, and caspase-12 was activated and cleaved, which suggested that these two pathways crucially contributed to hippocampal cell death. However, we found no changes in protein levels of phosphorylated JNK, implying that the JNK pathway was not the primary pathway involved in hippocampal apoptosis. It is more important that the cognitive impairments caused by RS were also effectively alleviated by salubrinal pretreatment. The present results suggested that ERS in hippocampus was excessively activated under stress, and amelioration of ERS could be a novel strategy to prevent and treat impaired cognitive function induced by RS.

1. Introduction

Stress is characterized by a combination of physiologic, neuroendocrine, behavioral, and emotional responses to novel or threatening stimuli [1]. Activation of the stress response leads to alterations that improve the ability of an organism to adjust its homeostasis and minimize the potential impact of a threat [2]. However, excessive stress can produce damaging physiological effects and is accompanied by various psychological and cognitive changes [3,4].

The hippocampus, which plays a vital role in learning and memory, contextual fear conditioning, and neuroendocrine regulation, is an important brain region susceptible to stress. It has been demonstrated

Abbreviations: ATF6, activating transcription factor 6; CHOP, C/EBP homologous protein; CNS, central nervous system; ER, endoplasmic reticulum; ERS, endoplasmic reticulum stress; GRP78, 78-kDa glucose-regulated protein; IRE1, inositol-requiring enzyme 1; JNK, c-JUN NH_2-terminal kinase; PERK, protein kinase RNA (PKR)-like ER kinase; RS, restraint stress; UPR, unfolded protein response; XBP-1, X-box binding protein 1.

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that chronic exposure to stress changes hippocampal function and structure [5,6] and reduces hippocampal volume [7]. Also, a reduction in the hippocampal volume has been reported in animal models of stressful events that have all been associated with memory loss in humans [8]. Furthermore, clinical studies have shown that individuals with depression suffer from hippocampal-dependent cognitive impairments and show reductions in hippocampal volume [9,10]. It is believed that the neuronal loss is one of the important factors involved in the reduction of hippocampal volume [11]. However, the underlying pathophysiological mechanisms of stress-induced hippocampal neuronal loss and cognitive impairments are complex and still not completely established.

Strong and long-lasting stress may result in the death or loss of neurons through apoptosis [12]. The endoplasmic reticulum (ER) is the primary site for secretory protein synthesis and maturation, Ca^{2+} storage and lipid biosynthesis. Various stimuli can disturb ER homeostasis and result in the accumulation of unfolded and misfolded proteins and pathological consequences, namely endoplasmic reticulum stress (ERS) [13]. Meanwhile, the accumulating unfolded proteins cause dissociation of 78-kDa glucose-regulated protein (GRP78) from the three major ER transmembrane effector proteins: protein kinase RNA (PKR)-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6) and a resultant launching of the unfolded protein response (UPR) [14]. Increased expression of GRP78 serves as a good marker for ERS [15]. Moderate ERS can relieve cellular dysfunction and enhance the chance for survival, but prolonged and/or severe stress leads to cell apoptosis. ERS is considered an early or initial response of cells under stress or damage and linked to neuronal death in various neurodegenerative diseases [16]. In these processes, ERS causes cell damage and apoptosis, and inhibiting ERS response pathways may provide nervous system protection.

Restraint stress (RS), a common animal model for the production of chronic stress, is one of the well accepted stressors used in experimental stress research, which elicits psychological frustration and physiological stress accompanied with vigorous struggle to escape [17]. In the present study, using the rat RS model for 21 consecutive days, we explored the effects of repeated RS on hippocampal apoptosis in vivo. Salubrinal, a selective inhibitor of eukaryotic translation initiation factor 2 subunit α (eIF2α) dephosphorylation, is known for its ability to protect cells from ERS-induced apoptosis [18], and pretreated by intracerebroventricular injection to examine the relationship between ERS and neuronal apoptosis, even cognitive impairments. Furthermore, the possible signaling pathways of ERS-induced apoptosis were assessed to clarify the mechanism of ERS-induced hippocampal apoptosis and cognitive impairments.

2. Materials and methods

2.1. Animals

All animal care and experimental protocols were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Institutional Animal Care and Use Committee of Hebei Medical University. Adult male Sprague–Dawley (SD) rats weighing 250–280 g were obtained from the Experimental Animal Center of Hebei Medical University (Shijiazhuang, Hebei, China). Every attempt was made to reduce the number and to minimize pain and suffering of animals.

2.2. Lateral ventricle cannula surgery and microinjections

After 7 days of acclimatization, all rats were housed individually in polypropylene cages under hygienic and standard environmental conditions (26 °C ± 2 °C, humidity 60%–70%, 12 h light/dark cycle). Surgical implantation of cannula was used for the intracerebroventricular injection and conducted in an aseptic environment. All rats were anesthetized with pentobarbital sodium (40 mg/kg, intraperitoneally) and placed in a stereotaxic apparatus (Benchmark Stereotaxic Instruments, USA). After the surface of the skull was exposed, a single hole was drilled through the skull above the left lateral ventricle (from the bregma to AP, 0.92 mm; ML, 1.65 mm). A stainless steel guide cannula was implanted 3.31 mm ventrally beneath the surface of the skull. To prevent occlusion, a dummy cannula was inserted into the guide cannula. Dental cement was used to fix the guide cannula to the skull. After surgery, all animals were treated with penicillin (1000 u/day i.m.) for 3 days and allowed to recover for at least 7 days.

Each microinjection was made with a 10 μl syringe (Hamilton, USA) attached to PE tubing connected to the injection cannula and was given at a rate of 0.5 μl/min in a volume of 2 μl using a syringe pump (KD Scientific, USA). The injection cannula extended 0.2 mm beyond the guide cannula and was left in place for 5 min following microinjections to minimize backflow of the drug.

2.3. Experimental design

All rats were divided into five groups: unstressed normal control group (Con group), restraint stress group (RS group), restraint stress combined with salubrinal (Sal, Santa Cruz Biotechnology, Santa Cruz, CA, USA) administration group (RS + Sal group), restraint stress combined with normal saline administration group (RS + NS group), and unstressed with salubrinal administration group (Sal group). Salubrinal (75 μM) or the same dose of normal saline respectively was injected into the lateral ventricle 30 min before restraint stress for 21 consecutive days, using the injection cannula.

In total, 86 animals were used. Nine rats were excluded because of anesthesia accident of surgery failure. At the end of all behavioral experiments, the location of the cannula was examined histologically. Due to the mislocation or dropout of cannula, 17 rats were not included in the statistical analyses. At last, 60 rats (12 rats per group) were used in the analyses.

Morris water maze test was assessed at 24 h after the last restraint stress. Then, the rats were decapitated in deep anesthesia, and brains were rapidly removed. The left hippocampus was dissected for apoptosis examination and the right was examined for protein or mRNA analysis.

2.4. Restraint stress procedure

The chronic repeated restraint stress protocol was adapted from the previous procedure [19]. And it was carried out by placing the rats in the restrainer (25 cm × 7 cm) without supplying food and water for 8 h (from 8:00 AM to 16:00 PM) every day for 21 consecutive days. The restraint devices had multiple air holes and allowed animals to stretch their legs, but not to move within the restrainers. The unstressed rats were left in their cages for the same time without food and water. During the rest period food and water were provided *ad libitum*.

2.5. Morris water maze test

The water maze consisted of a circular water tank (180 cm in diameter, 70 cm in height) that was partially filled with 24 °C ± 1 °C water. The pool was divided virtually into four equal quadrants labeled N-S-E-W. A colorless escape plat (10 cm in diameter) was hidden 1.5 cm below the surface of the water in a fixed location. The platform remained in the same quadrant during the entire experiment. The maze was located in a quiet test room, surrounded by many visual cues outside of the maze which was visible from within the pool and could be used by the rats for spatial orientation. The movement of the animals was recorded by a TV camera located over the center of the pool and was connected to a personal computer. The experiments were conducted two sessions per day for 5 days, each session comprised four trials, with an intertrial interval of 60 s, and the intersession interval was >2 h. In each trial, the