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Single-prolonged stress induce different change in the cell organelle of the hippocampal cells: A study of ultrastructure

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ABSTRACT

MRI studies have revealed structural and functional changes in the hippocampus of post-traumatic stress disorder (PTSD) patients. Previous studies conducted by us in a PTSD animal model found that single prolonged stress (SPS) induced abnormal morphological changes in hippocampal cells. The effects of SPS on cellular organelles of the hippocampal neurons remain unknown; however, these changes have been involved in SPS-induced abnormal hippocampal function. The aim of the present study is to examine ultrastructural changes in cellular organelles, including the lysosomes, mitochondria (Mit), Golgi apparatus, and endoplasmic reticulum (ER), following SPS exposure using transmission electron microscopy, enzyme histochemistry, and enzyme cytochemistry. First, morphological changes of the hippocampal cells and ultrastructural changes in cellular organelles, including lysosomes, ER, and Mit-induced by SPS were observed. Results from histo- and cytochemistry demonstrated that the Mit marker enzyme, cytochrome *c* oxidase (COX), and the lysosomal enzyme acid phosphatase, (ACP), increased following exposure to SPS. SPS induced COX release from Mit and led to a wider distribution of ACP in round lysosomes, NLY, and the Golgi. In addition, we found that SPS increased the presence of autophagosomes and induced changes in the autophagy-related protein, Beclin. These results indicated the differential effects of SPS on cellular organelles, that is, a positive effect on lysosomes as well as a negative effect on the Mit and ER. Increased lysosomal function may serve as protection against SPS-induced cell damage. Structural changes in the Mit and ER may be involved in SPS-induced disorders of energy metabolism and protein synthesis and export.

Introduction

Post-traumatic stress disorder (PTSD) is a stress-related cognitive and emotional disorder after exposure to a stressful or traumatic event, such as combat, an earthquake or violence (Adami et al., 2006; Liberzon and Martis, 2006). Neuroimaging studies have demonstrated post-traumatic stress disorder PTSD-induced abnormal function and structure in the hippocampus (Brown et al., 2003; Freeman et al., 1998; Mohanakrishnan et al., 2003; Villarreal et al., 2002). PTSD animal model- the single prolonged stress (SPS) model which was proposed by Liberzon et al. (1997, 1999) has been widely used. SPS animals are restrained for 2 h, subjected to a 20 min forced swim in 20–24 °C water and exposed to ether anesthesia. Rats exposed to SPS express enhanced negative feedback in their HPA axis and low levels of corticosterone in their plasma, a phenotype that resembles the neuroendocrine features of PTSD in humans (Liberzon et al., 1997, 1999). Previous studies by us have demonstrated abnormal morphology changes in the amygdala (Li et al., 2010), the raphe nucleus (Xie et al., 2012b) and the hippocampus (Han et al., 2013) of SPS rats, such as an existence of apoptositic body under electron microscopy (EM), But there have been no reports evaluating the *in vivo* effects of single prolonged stress (SPS) on cellular organelles, including mitochondria (Mit), rough endoplasmic reticulum (RER), and lysosomes. Therefore, the present work was conducted to investigate the *in vitro* effects of PTSD on cellular organelles.

Mitochondria (Mit) are the sites of aerobic respiration, and are thus central to energy production in eukaryotes. The number of Mit ranges from one to thousands per cell. They are usually positioned in cells nearest to the sites of energy utilization. Mit are characterized by a double membrane, inwardly projecting cristae and an internal matrix. The cristae greatly increase the surface area available for ATP synthesis and other reactions related to electron transport, the Krebs cycle, and oxidative phosphorylation.

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Furthermore, the matrix contains many enzymes needed for oxidation reactions of the Krebs cycle. In addition, the Mit sequester calcium and other divalent cations that are stored as small matrix granules.

Lysosomes are a heterogeneous collection of membrane-bound vesicles and vacuoles derived from the Golgi complex vesicles. They are usually spherical, ranging in diameter from 0.2 to 0.5 μm and contain more than 60 kinds of hydrolases. They are the intracellular digestive system for normal turnover and removal of useless organelles in the cells. In addition, in response to cell injury, they aid in the autolysis of cells. However, the presence of uniquely shaped lysosomes such as the long thread-like lysosome termed "nematolysosome" (NLY) in neurons has been reported (Shi et al., 1992). Acid phosphatase (ACP) is a marker enzyme of lysosomes and can be detected in irregular-shaped lysosomes and NLYs (Sakai et al., 1989). The Golgi complex is located near the cell nucleus consisting of flattened, membrane-bound sacs (cisternae) that can form a stack. Recently, the presence of the unique lysosome termed the "autophagosome" has been reported. A key role of autophagosomes is in the maintenance of energy homeostasis at the cellular level. Decreases in both volume and cell numbers of the hippocampus after PTSD exposure have been found in our previous studies. The degeneration of the hippocampus can be thought of as a failure to maintain cell survival and function in the brain. Further studies on lysosomes will therefore aid in the understanding of why PTSD induces apoptosis in the hippocampus.

The ER, in particular the RER, engages in the synthesis and export of proteins and glycoproteins. External ribosomes on the RER display a rough or granular appearance under EM. Ribosomes assemble polypeptides that are threaded into the cisternae lumina. The RER is the site of translation, folding, and transportation of newly formed proteins, which may then become part of cell membranes as integral membrane proteins and transmembrane receptors or be secreted by exocytosis. Secretory proteins are translocated into the ER lumen and subsequently transported to their destination. Furthermore, the ER is an essential intracellular organelle responsible for the synthesis and maturation of cell surface proteins as well as secretory proteins and is responsible for the maintenance of Ca^{2+} homeostasis (Wu and Kaufman, 2006; Ron and Walter, 2007). Many neurodegenerative disorders can cause the unfolded protein response (UPR) in the ER, thus leading to the accumulation of unfolded or misfolded proteins. If the stress on the ER is excessive or prolonged, the UPR will initiate the apoptotic cell death cascade (Nakagawa et al., 2000; Ferri and Kroemer, 2001). The objective of the UPR is to reduce the requirement for ER protein processing and to eliminate misfolded proteins as rapidly as possible. In previous studies, we found that PTSD induced the UPR in the hippocampus, amygdala, and PFC (Han et al., 2013; Xie et al., 2012a). Accumulation of unfolded proteins may affect hippocampal function, which may be associated with disorders of memory and learning in PTSD.

Our aim is to find out whether the abnormal alteration of the morphological structure of cell organelles. Exploration of hippocampal cell organelles after PTSD stimulation will enhance our knowledge of PTSD, potentially aiding in the understanding of PTSD-induced morphological and functional abnormal changes of the hippocampal cells.

Materials and methods

Animal model preparation and grouping

A total of 50 male Wistar rats (200–220 g) were maintained on a 12:12 h light/dark cycle, 19–21 °C room temperature, with access to ad libitum food and water. Animals were randomly divided into five groups: a control group, SPS groups examined on day 1 (1-day), day 4 (4-day), day 7 (7-day) and day 14 (14-day). All experimental procedures were approved by the ethics committee of China Medical University and conducted in accordance with the Guidelines Principles on Animal Experimentations for Laboratory Animal Science, China Medical University.

According to the method of Liberzon et al. (1997, 1999) rats were restrained for 2 h, immediately followed by 20 min of forced swimming in 20–24 °C water in a plastic tub (55.6 cm diameter, 45.4 cm height)which was filled two-thirds from the bottom. After a 15-min recuperation, rats were exposed to ether (using a dessicator) until general anesthesia, loss of toe and tail pinch responses, was induced (<5 min). Immediately after the induction of general anesthesia, rats were removed and placed in their home cages. The control rats remained in their home cages with no handling for 14 days and the SPS rats underwent the SPS procedure on the first day.

Transmission electron microscopy for the hippocampal cells

The animals were perfused through the heart with a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer. The brains were fixed in the same fixative solution for 24 h and post-fixed with 1% osmium tetroxide. After dehydration in ethanol with graded concentrations, the specimens were treated with propylene oxide and embedded in Epon 812. Semithin sections stained with 1% toluidine blue were examined in a light microscope, and suitable regions were carefully selected for trimming of the blocks. Ultrathin sections stained with uranyl acetate and lead citrate were examined in a transmission electron microscope (JEM-1 200EX; Jeol, Tokyo, Japan).

Enzyme histochemistry for ACPase

Wistar rats in each group are sequentially anesthetized with 2% pentobarbital by intraperitoneal injection of reinforced, open chest, applying cardiac perfusion, rapid processing hematogenous saline, 4% paraformaldehyde perfusion fixation, brains were removed, fixed in the same kind of fixing solution for 3 h, and immersed in Holt'S solution (40% sucrose, 0.1 mPBS preparation) for 2–3 d until they sunk. Brains were removed from Holt'S solution, with a cryostat microtome (model Leica) produced 14 μm thick frozen sections, the preparation of the ACPase incubation medium (according to Gomori'S method) (Araki and Ogawa, 1987; Araki et al., 1989): 50 mM sodium acetate buffer pH5.0 20 ml; Sucrose 1.6 g; 3% β-glycerophosphate 60 mg; the freshly prepared 1% lead nitrate was added dropwise 2 ml sealed (final PH5.0). Sections were placed above the incubation medium, 37 °C shaking water bath and incubated for 30 min. Partially sliced into β-glycerophosphate removal of the incubation medium to make the same reaction as a control, double distilled water, 1% amine curing coloring for 1–2 min, washed with distilled water, glycerogelatin were mounted, light microscopy (OLYMPUS) examination, radiography. For each rat, five sections were selected. We selected five images each section and analyzed the optical density (OD) of each ACPase positive cells in each image using the MetaMorph/DPIO/BX41 morphology image analysis system. And the average of OD was determined.

Enzyme cytochemistry for ACPase

After ACPase enzyme histocytochemical reaction, the sections were rinsed with 30 mM PIPES buffer containing 8% sucrose, and postfixed with 1% OsO4 in 30 mM PIPES buffer for 30 min at 4. The sections were again rinsed with double distilled water, dehydrated in graded alcohols, and embedded in Epon812 or super resin. Ultrathin sections (0.5–1.0 μm) were made with an AO ultratome