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Endoplasmic reticulum stress plays critical role in brain damage after chronic intermittent hypoxia in growing rats

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ABSTRACT

Obstructive sleep apnea hypopnea syndrome (OSAHS) in children is associated with multiple system morbidities. Cognitive dysfunction as a result of central nervous system complication has been reported in children with OSAHS. However, the underlying mechanisms are poorly understood. Endoplasmic reticulum stress (ERS)-related apoptosis plays an important role in various diseases of the central nervous system, but very little is known about the role of ERS in mediating pathophysiological reactions to cognitive dysfunction in OSAHS. Chronic intermittent hypoxia (CIH) exposures, modeling OSAHS, across 2 and 4 weeks in growing rats made more reference memory errors, working memory errors and total memory errors in the 8-Arm radial maze task, increased significantly TUNEL positive cells, upregulated the unfolded protein response in the hippocampus and prefrontal cortex as evidenced by increased phosphorylation of PKR-like endoplasmic reticulum kinase, inositol-requiring enzyme l and some downstream products. A selective inhibitor of eukaryotic initiation factor-2a dephosphorylation, salubrinal, prevented C/EBP-homologous protein activation in the hippocampus and prefrontal cortex throughout hypoxia/reoxygenation exposure. Our findings suggest that ERS mediated cell apoptosis may be one of the underlying mechanisms of cognitive dysfunction in OSAHS children. Further, a specific ERS inhibitor Salubrinal should be tested for neuroprotection against CIH-induced injury.

Introduction

Obstructive sleep apnea hypopnea syndrome (OSAHS), a condition characterized by repeated episodes of upper airway obstruction during sleep causes sleep apnea and hypopnea, associated with snore, disturbance in sleep architecture, frequent decreased blood oxygen saturation and excessive daytime sleepiness, and harmful to children's health and growth. It was reported that OSAHS damaged multiple systems in adult, in addition to the cardiovascular system, including respiratory system, digestive system, genitourinary system, endocrine system, and muscular system (Becker et al., 2003; Levy et al., 2012; Punjabi et al., 2002; Schober et al., 2011). Our previous study showed that nervous system injury is more common in OSAHS children and was associated with impaired cognitive functions (Cai et al., 2013). Cognitive injury in OSAHS patients may manifest in memory, learning, attention, concentration, judge, vigilance, or executive functions and damage psychomotor and general intelligence. Memory and learning are the most serious in children with OSAHS (Gozal et al., 2012; Hrubos-Strom et al., 2012; Owens, 2009), but the underlying mechanisms describing OSAHS-related cognitive impairment remain largely undefined. OSAHS is characterized by chronic intermittent hypoxia (CIH), it has been demonstrated that repetitive episodes of hypoxia and re-oxygenation play an important part in neurocognitive dysfunction (Halbower et al., 2006; Ward et al., 2009). Our previous study (Cai et al., 2010) suggested

Abbreviations: AI, Apoptotic Index; ASK1, Apoptosis signal regulating kinase1; ATF, Activating transcription factor; BiP, immunoglobulin-binding protein; CHOP, C/EBP-homologous protein; CIH, Chronic intermittent hypoxia; EDEM, ER degradation enhancing mannosidase like protein; eIF2a, eukaryotic translation initiation factor 2 subunit alpha; ER, Endoplasmic Reticulum; ERS, Endoplasmic Reticulum Stress; ERSE, ER stress response elements; IRE-1, Inositol-requiring enzyme l; JNK, c-Jun N-terminal kinase; OSAHS, Obstructive sleep apnea hypopnea syndrome; PERK, PRK-like ER kinase; RME, Reference memory error; TE, Total memory error; TRAF2, Tumor necrosis factor receptor-associated factor2; TUNEL, Terminaldeoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling; UPR, Unfolded protein response; WME, Working memory error; XBP-1, X-box-binding protein 1.

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that CIH exposure during a critical period of neuronal development can lead to substantial deficits in spatial memory in this model.

Recently, endoplasmic reticulum (ER), mediating cell stress responses have been linked to cell apoptosis. ER functions can be disturbed by different insults, such as accumulation of unfolded proteins and changes in calcium homeostasis (Boyce and Yuan, 2006; Verkhratsky, 2005). The unfolded protein reaction (UPR) signaling is orchestrated by three different pathways, each of which is initiated by an eponymous distinct sensor anchored in the ER as a transmembrane protein and termed the protein kinase dependent on RNA (PKR)-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE-1), and activating transcription factor 6 (ATF6) (Lupachyk et al., 2013). Disturbed ER functions induce expression of chaperones, attenuate protein translation, and activate ER-associated degradation (Kraskiewicz and FitzGerald, 2012). However, when this stress is insurmountable, the ER may take on the role of executioner, activating three pathways, including C/EBP-homologous protein (CHOP), c-Jun N-terminal kinase (JNK) and cysteine-containing gaspartate-specific proteases (caspase-12).

It has been demonstrated that ER played a central role in both adaptive responses to injury from ischemia–reperfusion challenges, and the change of hypoxia/reoxygenation in OSAHS is similar to that of ischemia–reperfusion. However, little is known about the role of ER responses in mediating pathophysiological reactions in OSAHS, so the aim of this study was to test the hypothesis that ER induced-damages to the hippocampus and prefrontal cortex in the growing rats play important roles in the pathogenesis of CIH associated neurocognitive dysfunction.

An adaptive response to endoplasmic reticulum stress (ERS) is the phosphorylation at Ser51 of the subunit alpha of eukaryotic initiation factor 2 (eIF2a), thereby blocking translation initiation and synthesis of membrane and secreted proteins critical in brain function. Induction of p-eIF2a is a key step in the ER stress response that will be demonstrated in this study by administration of salubrinal (Sal), a small molecule that increases p-eIF2a by inhibiting its dephosphorylation. In addition, we would like to confirm whether Sal could adapt inner-cellular stress to protect against ER stress-induced cell injury.

Materials and methods

Animal model of OSAHS and experimental groups

This study was approved by the Ethics Committee of Wenzhou Medical University. A total of sixty four male Sprague–Dawley rats (80–100 g; Shanghai Laboratory Animal Center, Shanghai, China) were randomly divided into eight groups that the number of each is eight by the method of random number table: 2-week-IH group (2IH), 4-week-IH group (4IH), 2-week-control group (2C), 4-week-control group (4C), 2-week-Salubrinal group (2SAL), 4-week-Salubrinal group (4SAL), 2-week-dimethylsulfoxide (DMSO) group (2DMSO), and 4-week-DMSO group (4DMSO). Rats except in control groups were laid in intermittent hypoxia cabin, an automated nitrogen/oxygen gas delivery system (Scientific Research Center of Wenzhou Medical College, Zhejiang, China) to deliver hypoxia/reoxygenation, using our previously described protocol (Cai et al., 2010). Two durations of chronic intermittent hypoxia (IH) (2 and 4 weeks) were studied.

Sal (Ellisville, USA) was initially solubilized in DMSO (Sigma, Helsinki, Finland) to make a 20 mM stock solution, which was diluted with buffered saline to 100 μM aliquots, also 0.48 mg of Sal in 1 ml of DMSO and 99 ml of buffered saline. The final concentration of DMSO was 1%. 100 μM Sal was administered at the dose of 1 mg/kg intraperitoneally 30 min before CIH daily (Sokka et al., 2007). An equal volume of DMSO (1%, 20.8 ml/kg/d) was injected in DMSO group rats.

Intermittent hypoxia exposure

The CIH model was established according to Wang et al. (2011) with modifications. A steel cabin (60 × 22 × 16 cm) for generating intermittent hypoxia and air control was created and an automated nitrogen/oxygen gas delivery system was used to deliver hypoxia/reoxygenation, using our previously described protocol (Cai et al., 2010). Briefly, in this system O_2 concentration could be reduced to a nadir of 9% ± 1.5% in 30 s by infusion of 99.99% nitrogen with the pressure kept at 0.3 KPa, stabilized at that level for 30 s, and then gradually increased to 21.0 ± 0.5% over the next 12 s by infusion of 99.50% oxygen (25 l/min) into the cabin. The computer controlled the infusion of oxygen and nitrogen. This cycle was repeated every 90 s over 7.5 h (from 8:00 to 15:30) during the animals' diurnal sleep period for certain days according to the experimental design. Ambient temperature was kept at 22–24 °C. The rats in IH group, SAL group and DMSO group were exposed to CIH for 2 weeks and 4 weeks. The control groups were placed in the cabin filled with compressed air for 2 weeks as 2C group or for 4 weeks as the 4C group respectively. The O_2 concentration was kept at 21.0 ± 0.5% in the control cabin.

Test of the chronic intermittent hypoxia cabin

The CIH cabin validation was performed before this experiment. 10 rats were anesthetized with 35 mg × kg^{-1} sodium pentobarbital (Sigma, USA) by intraperitoneal injection, and the carotid artery was catheterized. The catheter with heparin anticoagulation was inserted in the left carotid artery, sutured in place. When all rats recovered from surgery, 5 rats were selected randomly and placed individually into the IH cabin, while the left 5 rats were placed into the control cabin. Then, the experimental protocol was carried out for 2 h.

The arterial blood samples were drawn in 22.5 s interval during a single IH cycle, continuous blood for 5 times, every <3 s, with the initial nitrogen gas input as the first sample respectively. Arterial blood samples (0.5 ml) were collected in a 5-gauge needle at the end of each sequential condition and immediately analyzed using a blood-gas analyzer (GEM Premier 3000; America).

8-Arm (4-arm baited) radial maze test

The eight-Arm radial maze with four arm-baited was used to assess the spatial memory. We used a technique similar to that described in detail by Andoh et al. (2009) previously. The maze made of plexiglass was positioned in a testing room (room temperature of 25 °C, a humidity of 50 ± 5% and 12 h light/dark cycle). The central area was 30 cm in diameter and the arms were 50 cm long, 10 cm high, and 12 cm wide. A food cup (20 mm in diameter and 1 cm in depth) was located at the distal end of each arm. Prior to the experiments, the rats were restricted from chow for 2 days until their body weights were reduced to 80–85% of the baseline weight. First, an acclimation trial was conducted according to our previous experiment (Cai et al., 2010). After adaptation, all rats were trained once daily following CIH at 19:00 PM for 10 days. During the trial periods, 45 mg food pellets were located only in the food cups of arms 1, 3, 5 and 7 while the remaining 4 arms were empty. The rat was placed in an opaque box (20 × 20 × 20 cm) in the central platform. 15 s later, the box was taken away and the rat was permitted to seek food freely through the maze.

The performance of the rat was assessed by the number of error choices. A correct choice occurred if the rat entered into an unvisited arm, followed by food acquisition in the trial. An error was defined as the hind legs of the rats enter an incorrect arm. Entry into the never-baited arm was regarded as a reference memory error (RME), while re-entry into the arms in which the bait had already been acquired was considered as a working memory error (WME). RME plus WME was called as the total error (TE). The trial ended when the rat acquired