Behavioural Brain Research 296 (2016) 134–140

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Developmental loss of parvalbumin-positive cells in the prefrontal cortex and psychiatric anxiety after intermittent hypoxia exposures in neonatal rats might be mediated by NADPH oxidase-2

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HIGHLIGHTS

- Neonatal LTIH exposures increase NOX2-derived oxidative stress in the PFC.
- Neonatal LTIH exposures induce neurodevelopmental and psychiatric disorders.
- NOX2 is a core contributor to neonatal LTIH-induced neurodevelopmental and psychiatric disorders.

ARTICLE INFO

Article history:
Received 5 July 2015
Received in revised form 19 August 2015
Accepted 24 August 2015
Available online 28 August 2015

Keywords:
Neonatal rats
Intermittent hypoxia
NOX2-derived oxidative stress
Parvalbumin-positive cells
Psychiatric anxiety

ABSTRACT

Sleep apnea is more frequently experienced in neonatal life. Here we investigated the causal contribution of NOX2-derived oxidative stress in the prefrontal cortex (PFC) to neurodevelopmental alterations and psychiatric anxiety in a neonatal rat model of sleep apnea. Neonatal postnatal day 5 (P5) rats were exposed to long-term intermittent hypoxia (LTIH) or room air (RA) for 10 days. In the PFC, we determined the impact (I) of LTIH exposures on NADPH oxidase-2 (NOX2) expression and oxidative stress (II) of pharmacological NOX2 inhibition on LTIH-induced neurodevelopmental alterations in the P14 and P49 rats. Endpoints were NOX2-derived oxidative stress, parvalbumin (PV)-positive cells (PV-cells) and psychiatric anxiety. The results showed neonatal LTIH exposures increased NOX2 expression in the PFC of P14 rats, which was accompanied with elevation of NOX activity. Neonatal LTIH exposures increased oxidative stress in cortical PV-cells characterized by elevation of 8-hydroxy-20-deoxyguanosine (8-OHDG) level and reduced PV immunoreactivity, PV-cell counts in the PFC of P14 and P49 rats. Neonatal LTIH exposures increased psychiatric anxiety levels in the P49 rats. Pretreatment of neonatal rats before each neonatal LTIH exposure with the antioxidant/NOX inhibitor apocynin prevented the reduced PV immunoreactivity, PV-cells loss in the PFC and development of anxiety-like behavior. Our data suggest that NOX2-derived oxidative stress might be involved in the developmental loss of PV-cells in the PFC and development of psychiatric anxiety for neonatal rats exposed to LTIH.

1. Introduction

A substantial body of evidence has accumulated to indicate that sleep-disordered breathing is a growing health problem in both adult and pediatric populations. Obstructive sleep apnea (OSA) is a highly prevalent condition characterized by repeated episodes of intermittent hypoxia (IH) during sleep [1]. Among the vast spectrum of morbidities associated with OSA, neurocognitive deficits, anxiety and depression have emerged as prominent consequences of the disease. Development of rodent models mimicking components of OSA have yielded important insights into the mechanistic pathways underlying neural injury in the context of intermittent hypoxia, and have particularly pointed out the critical role of oxidative stress and inflammation-mediated neural injury in this context [2–6].

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http://dx.doi.org/10.1016/j.bbr.2015.08.033
0166-4328/

Intermittent hypoxia is more frequently experienced in neonatal life. Preterm birth occurs in high rate of pregnancies in the world. Premature infants experience hypoxic episodes due to immaturity of their respiratory and central nervous systems. These infants are at greater risk for short-term and long-term complications, including disabilities and impediments in growth and mental development. Apnea of prematurity profoundly affects brain development and results in cognitive impairments. Neonatal hypoxia in animals has been shown to induce neurodevelopmental dysfunctions in cortical interneurons during postnatal days and contribute to cognitive dysfunctions [7,8]. However, the effects and mechanisms of hypoxia on the developing brain and cognitive function are not well understood.

NOX-derived superoxide radical has been identified as a major contributor to oxidative injury in the brain under conditions of severe hypoxia and inflammation [9]. NOX has also been implicated in neurodegeneration, such as Alzheimer's and Parkinson's disease, and NOX is increasingly recognized for its role in health and disease, necessary for modulation of cell fate, neuronal activity [10] and cell signaling [9]. Long-term exposure to frequent hypoxia/reoxygenation events that mimic the altered oxygenation patterns of sleep-disordered breathing has been shown to induce NOX in selected brain regions, suggesting that activation of this enzyme may partly underlie the increased neuronal inflammation and oxidative stress observed in animal models of sleep-disordered breathing [11].

Prolonged oxidative injury is involved in the development of mental diseases [12], in particular, of schizophrenia-like disorders. Many hypotheses exist on the pathogenesis of these brain dysfunctions. Among them, a loss of phenotype of PV-cells, has been proposed [13,14]. Prolonged exposure to high doses of *N*-methyl-D-aspartate (NMDA) receptor antagonists (such as ketamine), which has been demonstrated to provoke psychotic symptoms in humans and behavioral alterations in rodents, also increases brain oxidative injury and results in a decrease of PV-cells in the PFC [15]. It has been suggested that the loss of PV-cells and change of GABAergic function may be a central feature in the pathogenesis of schizophrenia [15].

In the present study, we investigated the causal contribution of NOX2-derived oxidative stress in the PFC to neurodevelopmental alterations in a neonatal rat model of sleep apnea and tested the development of psychiatric anxiety for neonatal rats exposed to LTIH.

2. Materials and methods

2.1. Animals

The present study was approved by the Ethics Committee of Nanjing University, Nanjing, China, and was performed in accordance with the ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health (Bethesda, MD, USA). Pregnant Sprague-Dawley rats were purchased from the Model Animal Research Center of Nanjing University, Nanjing, China, and were housed individually in standard conditions with a 12-h light/dark cycle (light from 07:00 to 19:00) at 24 ± 1 °C and ad libitum access to food and water.

2.2. Neonatal long-term intermittent hypoxia exposures

Neonatal P5 male rat pups with 6.4–9.5 g body weight were culled and divided into two groups: long-term intermittent hypoxia group (LTIH group) and room air control group (RA group). The LTIH exposure window was selected as the period between P5 and P14 neonatal stages and the exposure time was controlled for 6 h per day. Briefly, rats were placed in a specialized chamber, which was flushed with alternating cycles of pure nitrogen and compressed standard room air (RA). The intermittent hypoxia profile consisted of alternating RA and nitrogen every 90 s. During hypoxia, inspired O_2 levels rapidly reached a nadir of 5% O_2. Oxygen concentration was continuously measured by an O_2 analyzer, and was changed by a computerized system controlling gas outlets. Control experiments were exposed to alternating cycles flushing room air every 90 s instead of nitrogen. The pups under either LTIH or RA exposures were nursed by a pseudo foster mother in a separate cage with homecage bedding for 6 h, and then stayed with their genetic mother for the rest of the day.

2.3. Drug treatment

We determined whether NOX2-derived oxidative stress is involved in the neurodevelopmental alterations in the PFC and development of psychiatric anxiety for neonatal rats exposed to LTIH. For the experiment, Rats were treated either with the antioxidant/NOX inhibitor apocynin (APO, Sigma, St. Louis, MO, USA; 4 mg/kg) or vehicle (VEH: 0.5% DMSO) by intraperitoneal (i.p.) injection 30 min before the exposure to 6 h daily for 10 days of LTIH.

2.4. Behavioral testing of psychiatric anxiety

Behavioral testing of psychiatric anxiety was carried out consecutively in the open-field test and elevated plus maze (EPM) at P49 and P50. The movement of each rat was monitored and analyzed using a computer-operated video tracking system. The apparatus was cleaned after each trial. All apparatus used in tests were come from Shanghai Soft maze Information Technology Co., Ltd., China. The rats after behavioral tests were used for biochemistry studies and would be administered euthanasia.

2.5. Open-field test

Emotional responses to the novel environment were measured by an open-field test. The open field task approaches the conflict between the innate fear that rodents have of the central area of a novel or brightly lit open field vs. their desire to explore new environments. When anxious, the natural tendency of rodents is to prefer staying close to the walls (thigmotaxis). In this context, anxiety-related behavior is measured by the degree to which rat avoid the center of the open field test. Rats were placed in an open-field (100 × 100 × 100 cm) chamber made of white acrylic for 10 min under 70 lux lighting conditions, in which the video tracking system quantifies the time spent in the center of the locomotion arena. Activity was measured as the total distance traveled.

2.6. EPM

The EPM test was used to assess psychiatric anxiety. The basic measure is the animal preference for dark, enclosed places over bright, exposed places. Rats prefer to enter into closed arms compared to open arms. Time spent in the dark area is viewed as avoidance or anxiety-like behavior. Rats were placed in the center of the maze facing a closed arm, and allowed to explore for 10 min in isolation. The following parameters were scored: (a) percent time spent in open arms; (b) the number of stretched-attend postures, defined as a posture in which the subject stretches forward and then retracts to its original position, performed from the central platform or enclosed-arms towards open-arms, was also recorded. This latter response is categorized as risk assessment, and has also been considered closed related to anxiety [16].