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Chronic asthma results in cognitive dysfunction in immature mice

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ABSTRACT

Asthma is the most common chronic childhood illness today. However, little attention is paid for the impacts of chronic asthma-induced hypoxia on cognitive function in children. The present study used immature mice to establish ovalbumin-induced chronic asthma model, and found that chronic asthma impaired learning and memory ability in Morris Water Maze test. Further study revealed that chronic asthma destroyed synaptic structure, impaired long-term potentiation (LTP) maintaining in the CA1 region of mouse hippocampal slices. We found that intermittent hypoxia during chronic asthma resulted in down-regulation of c-fos, Arc and neurogenesis, which was responsible for the impairment of learning and memory in immature mice. Moreover, our results showed that budesonide treatment alone was inadequate for attenuating chronic asthma-induced cognitive impairment. Therefore, our findings indicate that chronic asthma might result in cognitive dysfunction in children, and more attention should be paid for chronic asthma-induced brain damage in the clinical therapy.

Introduction

Asthma is the most common chronic childhood illness today (Yock et al., 2010). Both the number of children diagnosed with asthma and the severity of asthma have increased rapidly in recent years (Gozde et al., 2011). Asthma is an inflammatory disease that affects the airways. During an asthma attack, muscles that are around the airways tighten, which causes swelling of the airways' linings. The swelling allows less oxygen to be taken in by the body and used by vital organs. A long period of time without enough oxygen can affect brain function. Severe asthma can cause some degree of diffuse cerebral hypoxia (Brannan and Lougheed, 2012). If a child were to have a severe asthma attack and not receive adequate care in a certain window of time, the child could experience an anoxic insult including lack of oxygen to the brain (de Moraes et al., 2012).

Oxygen is vital to maintain the normal functions of almost all the organs, especially the brain which is one of the heaviest oxygen consumers in the body. The importance of oxygen to the brain is not only reflected in its development, but also depicted in various pathological processes of many cerebral diseases (Boroujerdi et al., 2012; Hummler et al., 2012). Decreases in oxygen supply to certain brain regions will result in memory impairments along with other deficits. Hence, a child could experience cognitive delay due to the lack of oxygen to the brain. There have been many studies focusing on the effects of stroke, trauma, and as well as sleep apnea syndrome on learning and memory (Cengiz et al., 2011; Dore-Duffy et al., 2011; Stowe et al., 2011). However, the effects of chronic asthma-induced intermittent hypoxia on cognition of children remain unclear. Therefore, the present study used immature mice to establish chronic asthma model, by which the impacts of asthma-induced brain hypoxia on learning and memory were investigated. Moreover, the mechanisms underlying chronic intermittent hypoxia on cognition were elucidated too.

Materials and methods

Animals

Twenty-to-22-day-old female BALB/c mice, weighing 12 g to 15 g, were obtained from the Experimental Animal Center of Jiangsu. Mice were housed with free access to food and water in a room with an ambient temperature of 22 ± 2 °C and a 12:12 h light/dark cycle. All experiments were carried out in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Mice were randomly assigned to three groups: control groups with saline treatment; asthmatic groups with saline treatment; asthmatic mice treated with budesonide (treatment details described as in the following).

Abbreviations: HIF-1α, hypoxia induced factor 1α; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; fEPSP, field excitatory postsynaptic potential; HFS, high-frequency stimulation; LTP, long-term potentiation; BALF, bronchial alveolar lavage fluid; HE, hematoxylin and eosin; PFA, paraformaldehyde; VEGF, vascular endothelial growth factor; GPR124, G protein-coupled receptor 124; MWM, Morris Water Maze; IEG, immediate early gene; DG, dentate gyrus.

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Sensitization and inhalational exposure

Allergic mouse models of asthma are generated by first sensitizing animals to a foreign protein, most commonly ovalbumin. Then, the animal receives a further antigen exposure either directly to the lungs in the form of an aerosol. This elicits an inflammatory reaction in the lungs characterized by an influx of eosinophils, epithelial thickening, and airway hyperresponsiveness (AHR). Mice were sensitized via 2 intraperitoneal injections of 10 μg of ovalbumin (grade V, ≥98% pure, Sigma, St. Louis, Missouri, USA) with alum adjuvant on days 0 and 14 of the experiment. Starting on day 21, the mice, housed in whole-body exposure chambers, were exposed to 1% aerosolized ovalbumin for 30 min a day, 3 days a week, for 9 weeks. Importantly, mice were exposed to 10% aerosolized ovalbumin for 30 min at the 9th, 18th and 24th times in order to aggravate condition. The temperature was kept at 20 °C to 25 °C and the relative humidity at 40% to 60%. An outline of the study procedures and time intervals is given in Fig. 1. The animals were subjected to Morris Water Maze test during the last week and were sacrificed for analysis on day 84.

Budesonide treatment

For mild chronic asthma, low-dose corticosteroids such as budesonide are recommended for preventing asthma symptoms due to its anti-inflammatory action. Micronized dry powder of budesonide was dissolved in 70% ethanol and diluted in sterile normal saline on the experimental day. Budesonide was given 1 h before OVA challenge or aggravation by intranasal administration (25 μl each time, 350 μg/kg) according to the treatment schedule in Fig. 1. Mice subjected to allergen challenge without budesonide treatment were treated with saline according to the treatment schedule in Fig. 1.

Airway inflammation analysis

The mice were anesthetized and placed in the supine position with the head tilted back, and then the trachea was cannulated. The lungs were lavaged three times with 0.3 ml of sterile PBS. The bronchial alveolar lavage fluid (BALF) was immediately centrifuged (5 min, 4 °C, 151 g/min). Cell pellets were resuspended in 1 ml PBS for total and differential cell counting. Differential cell counting was performed on hematoxylin and eosin (HE)-stained cytospins. On each cytospin 200 to 500 cells were counted. Subsequent to lavage, the lungs were isolated and instilled with 0.4 ml of 4% paraformaldehyde (PFA) and placed in PFA overnight for histology. PFA-fixed lung sections were stained with HE stain. Six to eight slices per mouse were used for evaluation.

Morris Water Maze

The water maze consisted of a black pool (100 cm in diameter, 75 cm high, bottom 45 cm above floor level) filled with water (opaque with ink) and a black platform (10 cm in diameter, 50 cm high) submerged 1 cm below the water surface. The water was maintained at 22 ± 2 °C, and the platform was placed in either of four virtual quadrants at 20 cm from the sidewall. The movements of mice were recorded with a video camera connected to a computer. Data were analyzed using a tracking program (version 2.5.2, XinTianDi Technology, Beijing, China). Tests were conducted between 0800 and 1300 h. One day before training, the mice were allowed to swim for 2 min. For learning, the offspring were given three trials on each day for 4 consecutive days. Each mouse was placed at one of the other three starting points that were used in a pseudorandom order so that each position was used once in each block of three trials. If the mice failed to find the escape platform within 60 s, the researcher would guide them to the platform where they were allowed to remain for 10 s. A 1-h interval was imposed before the beginning of the next trial. The platform location was not changed during the learning period. The amount of time the mice spent in looking for the submerged platform (escape latency) was recorded. One day after the learning period, the animals were subjected to a single 60-s probe test in which the platform was removed from the pool. The length of time that mice took to find and stayed at the designated quadrant was analyzed.

In vitro electrophysiology

The hippocampus was removed from the brains and sliced at 400 μm using a vibratome, and then placed in a holding chamber for 1 h at room temperature. Three slices from each mouse were then transferred to the recording chamber. The slices were maintained at 33.5 ± 0.5 °C and continuously superfused with artificial cerebrospinal fluid (ACSF, 1.3–1.5 ml/min) that had been saturated with 95% O_2 and 5% CO_2 mixed gas. The composition of the ACSF (pH 7.4) is (in mM): 127 NaCl, 4.7 KCl, 12 NaH_2PO_4, 12 $MgCl_2$, 2.5 $CaCl_2$, 220 $NaHCO_3$, and 10 mM glucose. Field excitatory postsynaptic potential (fEPSP) of CA1 was recorded from the stratum pyramidale with a glass pipette filled with 2% pontamine sky blue and 0.5 M sodium acetate. The stimulating electrode was placed on a Schaffer collateral of CA3. A single test stimulus (200 s) was applied at intervals of 1 s and the stimulus intensity was set at a level when a population spike of 60–70% of the maximum was evoked. High-frequency stimulation (HFS) (100 Hz, 100 pulses) was applied to induce long-term potentiation (LTP). Before the induction of LTP, a baseline fEPSP was recorded for 20 min.

Electron microscopy

One mm^3 CA3 from the right hippocampus of the three groups was dissected. The tissue was fixed in 2.5% glutaraldehyde for 1 h. After being washed, sections were post-fixed in a solution of 1% osmium tetroxide for 1 h, dehydrated through a graded series of ethanol, and embedded in an admixture of acetone and epon resin (1:1) for 2 h, and then in epon resin for 2 h. Blocks were polymerized at 60 °C for 48 h. The pyramidal layer of the CA3 was selected precisely

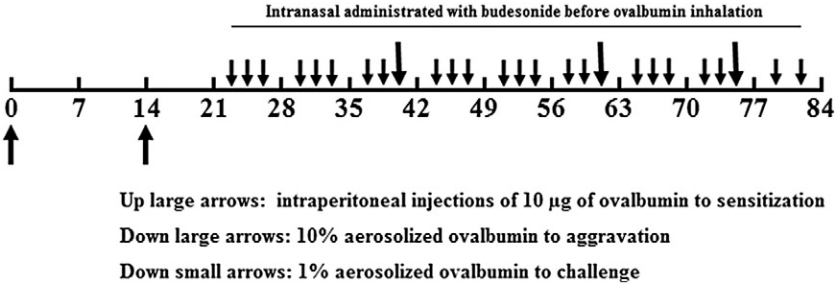

Fig. 1. Procedures for establishing chronic asthma models. The down small arrows indicated mice were exposed to 1% aerosolized ovalbumin to challenge; the down large arrows indicated that the mice were exposed to 10% aerosolized ovalbumin to aggravation; the up large arrows indicated the mice were sensitized via intraperitoneal injections of 10 μg of ovalbumin; budesonide was given 1 h before OVA challenge or aggravation by intranasal administration.