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Neuroprotective effects of therapeutic hypercapnia on spatial memory and sensorimotor impairment via anti-apoptotic mechanisms after focal cerebral ischemia/reperfusion

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HIGHLIGHTS

- Hypercapnia attenuates neuronal apoptosis caused by stroke.
- Hypercapnia improves impaired spatial memory and sensorimotor.
- Hypercapnia exerts neuroprotection through anti-apoptotic mechanisms.

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ABSTRACT

A number of studies have demonstrated that therapeutic hypercapnia ameliorates neurological deficits and attenuates the histological damage of cerebral ischemia-reperfusion injury. However, the effects of therapeutic hypercapnia on impaired spatial memory have not been reported. Here we aimed to investigate the effects and mechanisms of therapeutic hypercapnia on spatial memory in a rat model of focal cerebral ischemia induced by middle cerebral artery occlusion/reperfusion (MCAO/R). Adult male rats were randomly assigned into three experimental groups: sham (sham operation), IR (MCAO/R), and hypercapnia [arterial blood CO_2 tension ($PaCO_2$) 80–100 mmHg + IR] groups. Sensorimotor deficits and spatial memory testing were evaluated by an 18-point scoring system and an 8-arm radial maze task, respectively. The hippocampal histological damage and the percentage of apoptotic neurons were evaluated by hematoxylin and eosin (HE) staining as well as flow cytometry. Western blotting was used to investigate the changes of the apoptosis-related Bcl-2 and Bax proteins. The results indicated that hypercapnia treatment significantly improved the abilities of impaired sensorimotor and spatial memory after MCAO/R. Moreover, hypercapnia treatment significantly increased the percentage of surviving neurons and decreased the percentage of apoptotic neurons in the hippocampus after MCAO/R damage. The expressions of anti-apoptotic protein Bcl-2 and pro-apoptotic protein Bax were significantly increased and decreased in the hypercapnia treated rats, respectively. These findings suggest that therapeutic hypercapnia can attenuate neuronal apoptosis and improve impaired spatial memory and sensorimotor after MCAO/R, which may be attributable to its anti-apoptotic effects through modulation of apoptosis-related protein.

1. Introduction

Ischemic brain injury, including stroke, is one of the leading causes of long-term disability and mortality and is being increasingly recognized as a significant disorder that threatens human health and life [1–3]. Ischemic brain injury often accompanies neuronal dysfunction and cell death, particularly in hippocampal pyramidal neurons, which is correlated with spatial memory [4].

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Although ischemia-induced memory deficits cause high morbidity in clinical cases, and despite extensive research, few neuroprotectants to date have been successfully developed from bench to bedside.

Therapeutic hypercapnia is induced by adding carbon dioxide (CO_2) to inspired gas and has been regarded as an effective strategy for lung, myocardial, intestinal, and central nervous system ischemic injury models [5–7]. Our previous studies suggest that therapeutic hypercapnia [arterial blood CO_2 tension ($PaCO_2$) 80–100 mmHg] ameliorates neurological deficits and attenuates histological damage in both focal and global cerebral ischemia-reperfusion injury models [8,9]. However, the effects and mechanisms of therapeutic hypercapnia on impaired spatial memory in a focal cerebral ischemia model have not been reported. In addition, many studies have shown that variations of apoptosis-related proteins, Bcl-2 and Bax, play an important role in neuronal death after MCAO/R [10,11]. Thus, the aim of this study was to evaluate the effects of therapeutic hypercapnia on impaired spatial memory and sensorimotor dysfunction after MCAO/R in rats and to explore whether this hypercapnia-induced neuroprotection is modulated by apoptosis-related proteins.

2. Materials and methods

Please see details in the supplementary material.

2.1. Experimental animals

Adult male Sprague Dawley rats weighing 250–280 g were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, PR China). All animal experiments were approved by the guidelines of Institute for Laboratory Animal Research of Harbin Medical University and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996, USA).

2.2. Experimental protocol

Eighty-four rats were randomly assigned into 3 experimental groups: sham group (sham operation, $n = 28$), IR group (MCAO/R, $n = 28$), and hypercapnia group ($PaCO_2$ 80–100 mmHg + IR, $n = 28$). The rats in the sham group underwent left common carotid artery (CCA) and external carotid artery (ECA) exposure without middle cerebral artery (MCA) occlusion, followed by inhalation of 30% O_2 for 2 h. In the IR group, the MCA was occluded for 90 min under the same anesthetic conditions and surgical procedures as the Sham group, followed by reperfusion and inhalation of 30% O_2 for 2 h. In the hypercapnia group, rats received the same IR protocol and the same fraction of inspired oxygen as described for the IR group, with hypercapnia applied at the beginning of reperfusion. Animals were tested at 24 h, 72 h, 7 days, 14 days, 21 days, and 28 days to evaluate spatial memory, and sensorimotor function. Twenty-four hours after reperfusion, a subset of the rats was sacrificed and their brains were quickly harvested for hematoxylin and eosin (HE) staining, western blotting, and flow cytometry (The experimental protocol is shown in Fig. 1A).

2.3. MCAO/R model

MCAO/R rats were generated as previously described [9]. Regional cerebral blood flow (rCBF) was monitored by laser Doppler flowmeter (PeriFlux system 5000, Preimed AB, China).

2.4. Therapeutic hypercapnia

Therapeutic hypercapnia was performed as described previously [9]. Physiologic variables were recorded at five time points including after 90 min of ischemia and every 30 min after the start of reperfusion (reperfusion 30, 60, 90, and 120 min).

2.5. Sensorimotor scoring

A blinded observer evaluated sensorimotor scores at every designated time-point using a scoring system utilized an 18-point scale as described previously [12].

2.6. Radial arm maze

Spatial memory testing was carried out using an 8-arm radial maze task (Xinruan Information Technology Co. Ltd., Shanghai, China), as previously reported [13]. Rats were randomly allocated to one of three experimental groups and were tested for spatial memory on days 1, 3, 7, 14, 21, 28 by a blinded observer and reference memory error, working memory error, and distance travelled were recorded.

2.7. Histological examination

At 24 h after reperfusion, rats were deeply anesthetized and decapitated, and the brains were removed, and embedded in paraffin. Paraffin-embedded tissues were sectioned at 5 μm at the level of the hippocampus according to standard procedures. The sections were processed for HE staining, which was used to count the number of dead cells in the hippocampus. The density of surviving neurons was expressed as the number of cells per mm length measured along the hippocampal CA1 pyramidal layer.

2.8. Analysis of apoptosis by flow cytometry

Apoptosis was assessed by an Annexin V-FITC/propidium iodide (PI) dual staining kit (556547, FITC Annexin V Apoptosis Detection Kit I, BD Biosciences, USA) according to the manufacturer's instructions. Analyses were performed with Cell Quest Pro software (BD Biosciences, San Jose, CA) and flow cytometry (BD FACSCalibur™ Flow Cytometry, BD Biosciences, USA.). For each sample, data from 10,000 cells were recorded in list mode on logarithmic scales. There are four quadrants represent dead neurons, late apoptotic neurons, normal neurons, early apoptotic neurons, respectively in flow cytometry results. The percentage of apoptotic cell was calculated using 100 × (early + late) apoptotic neurons number/total neurons number.

2.9. Western blot analysis

Twenty-four hours after surgery, rats were sacrificed under deep anesthesia. Hippocampal samples were stored at −80 °C until analysis, when western blot and protein extraction were performed as described previously [14]. The primary antibodies were against Bcl-2, Bax (1:1000, Cell Signaling Technology Inc., Danvers, MA, USA) and secondary antibodies (1:5000, Rockland Inc., Gilbertsville, PA, USA). Blot bands were densitometrically quantified with Image J software (v1.33, NIH, Bethesda, MD, USA). β-Actin (1:1000, ZSGB-Bio, Beijing, China) was blotted on the same membrane as a loading control. The quantified values were expressed as a percentage of sham (100%).