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Original Article

Relationship of learning and memory impairment in chronic intermittent hypoxic rats with hippocampus ERK activation and microtubule-associated protein 2 expression

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Abstract: Objective: To investigate the relationship of the learning and memory function with ERK signaling pathway changes and microtubule-associated protein-2 (MAP-2) expression in hippocampal CA1 region of intermittent hypoxic rats. Methods: The chronic intermittent hypoxia rat model was established; 96 mature male wistar rats were randomly divided into three groups: normal air (control) group, 5% intermittent hypoxia (5% IH) group and the intervention (5% IH U0126) group; homemade hypoxic chamber was used to simulate 5% chronic intermittent hypoxia model; exposure time was 8 h/d, continuing 28 d; after modeling, learning and memory behaviors of the rats were detected by Morris water maze test at four time points (7, 14, 21, 28 d); P-ERK1/2 and MAP-2 protein expression in hippocampal CA1 region was detected by immunohistochemistry. Results: Compared with the control group, escape latencies at 14, 21, 28 d in 5% IH group were increased significantly ($P<0.05$); from 14 d, with prolonged hypoxia time, escape latency prolonged ($P<0.05$); and the time of rats crossing the target quadrant was shortened ($P<0.05$). In the intervention group escape latency time at 7, 14, 21, 28 d was similar to the 5% IH group. Compared with the intervention group, 5% IH group changed more obviously ($P<0.05$). Immunohistochemistry results showed that, in 5% IH group, P-ERK1/2 protein expression levels in hippocampal CA1 region at 7, 14, 21, 28 d were significantly higher than those in control group ($P<0.05$). In the intervention group, P-ERK1/2 protein expression levels at 7, 14, 21, 28 d showed no significant difference compared with the control group ($P>0.05$). However, compared to control group, MAP-2 protein expression levels were reduced significantly both in 5% IH group and in the intervention group. Conclusion: Chronic intermittent hypoxia can lead to learning and memory dysfunction in rats, activate ERK pathway in rat hippocampus, and induce MAP-2 protein degradation. Chronic intermittent hypoxia leading to reduced levels of learning and memory in rats may be associated with reduced MAP-2 levels in the hippocampus.

Keywords: Obstructive sleep apnea hypopnea syndrome, chronic intermittent hypoxia, cognition, P-ERK1/2, MAP-2

Introduction

Obstructive sleep apnea hypopnea syndrome (OSAHS) is a chronic respiratory disease with clinical characteristics of repeated complete or incomplete upper airway obstruction in sleep state, accompanied by intermittent hypoxemia, sleep structure disorders and (or) merger hypercapnia. OSAHS is now being considered as an independent risk factor of cardiovascular disease, nerve damage and dysfunction of metabolism, while the main clinical manifestation of nervous system damage is cognitive dysfunction, and the removal of obstructive cause cannot make complete reversal in cognitive dysfunction [1], seriously affecting the health and quality of life of patients. Extracellular signaling kinase (ERK) mitogen is a member of mitogen-activated protein kinases (MAPKs) family; a large number of studies have demonstrated that ERK signaling pathway is closely related to learning and memory processes [2]. Microtubule-associated protein-2 (MAP-2) is a protein related with neuron regeneration and repair; it is a molecular marker of neuronal regeneration, with neuron specificity [3]. In this

Table 1. Comparison of the rat escape latency (s, $\bar{x}\pm s$)

Group	7 d (n=8)	14 d (n=8)	21 d (n=8)	28 d (n=8)
Control group	23.26 ± 1.30	21.14 ± 2.87	23.20 ± 2.86	21.47 ± 3.04
Intervention group	21.36 ± 2.92	26.62 ± 3.22$^{▲}$	31.32 ± 4.54$^{▲}$	40.55 ± 3.95$^{▲,*,•}$
5% IH group	21.79 ± 3.36	34.56 ± 2.41$^{▲,▼}$	45.66 ± 3.89$^{▲,*,▼}$	58.93 ± 3.63$^{▲,*,•,▼}$

Note: Compared with the control group, $^{▲}$P<0.05; compared with the intervention group, $^{▼}$P<0.05; within the intervention group and 5% IH group: Compared with 7 d, $^{▲}$P<0.05; compared with 14 d, *P<0.05; compared with 21 d, $^{•}$P<0.05.

Table 2. Comparison of the time of rats crossing the target quadrant (s, $\bar{x}\pm s$)

Group	7 d (n=8)	14 d (n=8)	21 d (n=8)	28 d (n=8)
Control group	53.16 ± 4.75	52.29 ± 4.93	49.22 ± 5.35	50.49 ± 3.40
Intervention group	53.06 ± 2.84	44.21 ± 3.65$^{▲}$	40.38 ± 4.38$^{▲}$	32.33 ± 5.34$^{▲,*,•}$
5% IH group	50.15 ± 3.90	39.23 ± 3.57$^{▲,▼}$	29.40 ± 3.86$^{▲,*,▼}$	22.95 ± 2.26$^{▲,*,•,▼}$

Note: Compared with the control group, $^{▲}$P<0.05; compared with the intervention group, $^{▼}$P<0.05; within the intervention group and 5% IH group: Compared with 7 d, $^{▲}$P<0.05; compared with 14 d, *P<0.05; compared with 21 d, $^{•}$P<0.05.

Table 3. P-ERK1/2 protein immunohistochemical results in hippocampal CA1 region of rats (IOD, $\bar{x}\pm s$)

Group	7 d (n=8)	14 d (n=8)	21 d (n=8)	28 d (n=8)
Control group	8.40 ± 3.15	8.21 ± 3.49	8.88 ± 2.82	7.43 ± 3.26
Intervention group	7.42 ± 3.16	9.17 ± 3.42	9.42 ± 3.07	8.90 ± 3.72
5% IH group	31.31 ± 3.96$^{*,•,▼}$	21.30 ± 2.83$^{▲,▼,•}$	43.04 ± 5.58$^{▲,*,▼}$	55.03 ± 4.08$^{▲,*,•,▼}$

Note: Compared with the control group, $^{▲}$P<0.05; compared with the intervention group, $^{▼}$P<0.05; within the intervention group and 5% IH group: Compared with 7 d, $^{▲}$P<0.05; compared with 14 d, *P<0.05; compared with 21 d, $^{•}$P<0.05.

study, through the establishment of chronic intermittent hypoxia rat model to simulate OSAHS hypoxia-reoxygenation process, learning and memory function changes, hippocampal CA1 P-ERK1/2 and MAP-2 expression and changes after the intervention of ERK inhibitor U0126 were observed in each group to further explore the relevant mechanisms of cognitive impairment in patients with OSAHS, providing more effective treatment to avoid complications in patients with OSAHS.

Materials and method

Experimental materials

Experimental animals: 96 adult male Wistar rats (Tianjin Shanchuanhong Laboratory Animal Science and Technology Co., Ltd.), license number: SCXK (Tianjin) 2009-0001, body weight 180 ± 10 g, with free access to food and water in conventional breeding box.

Main reagents and instruments: P-ERK1/2 rabbit anti-mouse polyclonal antibody (Cell signaling company, USA); MAP-2 rabbit anti-mouse monoclonal antibody (Beijing Biosynthesis Biotechnology Co., Ltd.); U0126 (Germany Leika company); low oxygen chamber (Changsha ChangJin Technology Co., Ltd.); hypoxia process control and monitoring system (Tangshan Friendship Technology Co., Ltd.); Morris water maze system (Institute of Materia Medica, Chinese Academy of Medical Sciences).

Experimental method

Animal grouping and preparation of model: The rats were randomly divided into the control group, 5% ZH group, and the intervention group, n=32; each group was divided into 7 d, 14 d, 21 d and 28 d groups, n=8. Every day the rats were exposed in the cabin model for 8 hours; the control group: compressed air was continuously injected into the cabin to maintain oxygen concentration at 21%. Intermittent hypoxia group: nitrogen was injected into the cabin for 30 s; when oxygen concentration was as low as 5%, the air was injected into the cabin for 40 s to increase oxygen concentration to 21%, and then the air was injected into the cabin for 50 s to maintain oxygen concentration at 21%; per 2 min was a cycle. Intervention group: In 30 min before placed into hypoxia cabin, U0126 intravenous injection (0.2 mg/kg) was performed; hypoxia condition was the same as intermittent

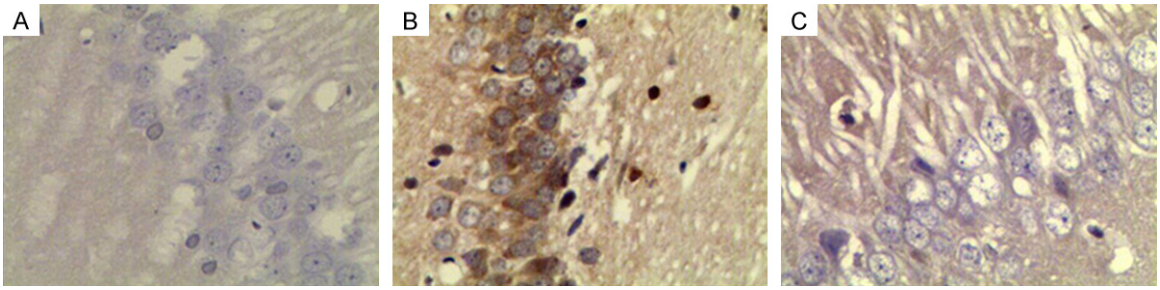

Figure 1. P-ERK1/2 protein expression in hippocampal CA1 region of rats. SP method, × 400. A: Control group; B: 5% IH 28 d group; C: Intervention 28 d group.

Table 4. MAP-2 protein immunohistochemical results in hippocampal CA1 region of rats (IOD, $\bar{x}\pm s$)

Group	7 d (n=8)	14 d (n=8)	21 d (n=8)	28 d (n=8)
Control group	68.12 ± 5.04	70.24 ± 2.67	71.99 ± 5.59	67.49 ± 4.39
Intervention group	71.24 ± 5.43	61.20 ± 3.66$^{▲}$	50.81 ± 4.87$^{▲,*}$	42.06 ± 4.40$^{▲,*,•}$
5% IH group	68.70 ± 6.60	49.06 ± 5.03$^{▲,★}$	38.98 ± 5.72$^{▲,*,★}$	30.23 ± 4.49$^{▲,*,•,★}$

Note: Compared with the control group, $^{▲}$P<0.05; compared with the intervention group, $^{★}$P<0.05; within the intervention group and 5% IH group: Compared with 7 d, $^{▲}$P<0.05; compared with 14 d, *P<0.05; compared with 21 d, $^{•}$P<0.05.

hypoxia group. At 7, 14, 21, and 28 days, Morris water maze test and immunohistochemistry index detection were performed.

Morris water maze was used to test learning and memory function of rats: According to the method of Ref. [4], the Morris water maze test was performed. In morning, the animals were subjected to 5 times of escape lantency and crossing the target quadrant exercise, and test was performed each six times in the morning and in the afternoon, respectively; escape latency time and across target quadrant time were recorded in each group; the average of the detected record was calculated.

Immunohistochemistry was used to detect P-ERK1/2 and MAP-2 expression: After the simulation, brain tissue of the rats in each group was removed at a predetermined time point for biopsy; SP immunohistochemical staining was performed, and positive cells were observed; Motic medical image analysis system immunohistochemical analysis module was used to calculate IOD values of positive target with the same area.

Statistical analysis

SPSS17.0 statistical analysis software was used to analyze data; data ($\bar{x}$ ± s) were analyzed by ANOVA; pairwise comparisons were performed using (LSD) method; P<0.05 indicated a significant difference.

Results

Place navigation test

In each group, on the 5th day of training, rat escape latency was significantly reduced comparison with the first day; the difference between different time points was not significant in the control group (P>0.05); in the intervention group and 5% IH group, with hypoxia prolonged, escape latency time was gradually extended (P<0.05); compared with the intervention group, the changes in 5% IH group were more obvious (P<0.05), shown in **Table 1**.

Space exploration experiments

With hypoxia was gradually extended, the time of rats across the target quadrant did not change significantly in control group (P>0.05); compared with the control group, the time of rats crossing the target quadrant was significantly shorter in the intervention group and 5% IH group (P<0.05); compared with the intervention group, 5% IH group changed more obviously (P<0.05), shown in **Table 2**.

Effects of intermittent hypoxia on expression of P-ERK1/2 in hippocampal neurons: Under an optical microscope, brownish yellow or pale yel-

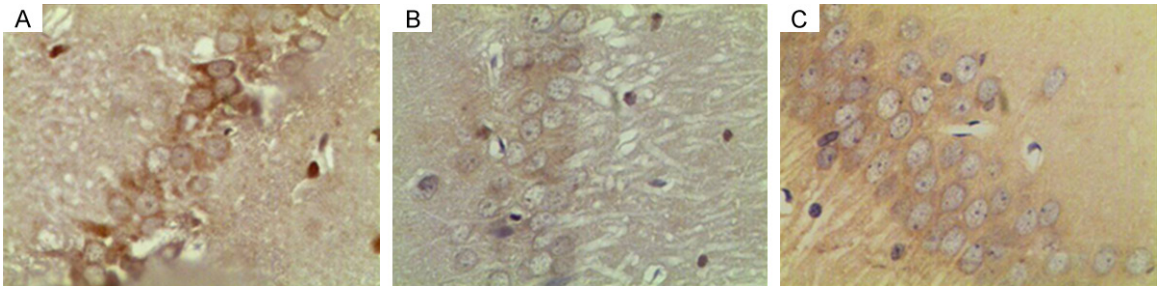

Figure 2. MAP-2 protein expression in hippocampal CA1 region of rats SP method, × 400. A: Control group; B: 5% IH 28 d group; C: Intervention 28 d group.

low in the cytoplasm or nucleus was positive. Scattered positive expressing cells were observed in the control group and the intervention group; in 5% ZH group positive expression in hippocampus CAI region was significantly higher than that in the intervention group (P<0.05), shown in **Table 3**; **Figure 1**.

Effects of intermittent hypoxia on expression of MAP-2 in hippocampal neurons: Under an optical microscope, brownish yellow or pale yellow in the cytoplasm or nucleus was positive. The control group showed high expression; positive expression in 5% ZH group and intervention group was significantly lower than that in the control group (P<0.05), shown in **Table 4**; **Figure 2**.

Discussion

Sleep apnea hypopnea syndrome is a chronic sleep breathing disease characterized by recurrent hypoxia/reoxygenation in sleep state. This study used the rat models of chronic intermittent hypoxia at different time points to simulate human OSAHS intermittent hypoxia; the results show that intermittent hypoxia can directly cause cognitive decline in rats, and the decline extent was related with duration of hypoxia, consistent with clinical practice [5].

ERK1/2 is an important serine/threonine protein kinase; its substrates include histones, transcription factors, K + channels and other intracellular kinases; these substances as important components of cells, are indispensable in learning and memory formation process. Numerous studies have confirmed that, ERK pathway is closely related to learning and memory injury [2], but its mechanism of action remains controversial. Some studies suggest that activation of the ERK pathway is advantageous in learning and memory formation process; For example, in item identification experiments, the researchers injected MEK inhibitor in mouse nose cortical to block the activation of ERK pathway, and a significant reduction was observed in the ability to identify objects [6]. In the hippocampus of seizure rats, the expression of ERK protein significantly decreased, and Morris water maze test showed that learning and memory ability of rats significantly decreased [7]. While other studies [8] found that ERK1/2 activation also promote apoptosis, which led to nerve cell apoptosis in models of ischemia-reperfusion and cerebral trauma due to oxidative stress, toxic substances or lack of growth factors and other stimulation. The results of this study showed that, with time, in 5% ZH group, phosphorylated ERK positive cells in the hippocampus of rats gradually increased, while the U0126 intervention group and the control group had no significant changes, which was consistent with rat behavior trends, indicating that ERK activation is involved in the pathogenesis of chronic intermittent hypoxia nerve injury. We also noted that, in 5% IH group, P-ERK1/2 expression had a trough value in this experiment; it showed a downward trend on d14 but was still significantly higher than control group; then on d21 and d28 d, the expression showed an increasing trend, which may indicate that self-regulatory mechanisms of ERK pathway were working. Some studies [9, 10] showed that self-regulatory mechanisms of ERK signaling pathway included positive feedback regulation and negative feedback regulation, and the main factor determining ERK signal output was the duration of the signal; the present results suggest that ERK pathway may be gradually extended with hypoxia time, which may be through self-regulatory mechanisms to activate downstream gene expression to par-

ticipate in nervous system lesion formation in early chronic intermittent hypoxia.

In recent years, several studies have found that in cerebral ischemia and reperfusion, experimental brain trauma and other neuronal degeneration processes, intracellular calcium homeostasis disruption leads to an increase in calcium influx and calcium overload, resulting in the activation of calcium neutral protease-calpain; after activation, calpain can act on cytoskeletal proteins (MAP-2, etc.) to cause destruction, degradation and loss of cytoskeletal proteins, leading to axonal transport barriers and damage to cell structural integrity, eventually resulting in neuronal degeneration and necrosis, and nerve function disorders [11]. Calpain is a direct substrate of ERK1/2; ERK1/2 activation can increase calpain activity [12], so presumably, ERK pathway can indirectly activate calpain to degrade MAP-2, leading to nervous system dysfunction.

MAP-2 is highly expressed in the central nervous system and involved in neuronal development, structural stability, projection formation and synaptic plasticity; it can promote neuronal regeneration and repair, sensitive to a variety of factors and ischemic injury, which can be used as an early marker of neuronal morphology damage. Studies have shown that chronic stress can lead to decreased MAP-2 expression in rat hippocampus and neuronal structural damage, so that the cognitive function was significantly reduced in rats [13]; while in the rat model of vascular dementia, it had been found that water maze score of experimental animals was related with MAP-2 expression in hippocampal CA3 region [14]. This study showed that in intermittent hypoxia group, with time prolonged, hypoxia MAP-2 expression decreased, consistent with the trend of rat behavior changes, suggesting that long-term intermittent hypoxia can lead to the degradation of MAP-2, resulting in learning and memory behavioral disorders. The MAP-2 in the intervention group was significantly higher than that in hypoxia group; learning and memory function of rats also recovered, suggesting that MAP-2 degradation was associated with ERK pathway activation. In addition, this study showed that between the intervention and control groups, there was no significant difference in P-ERK positive expression in hippocampus of rats, while between the intervention group and 5% IH group and control group, there were differences in cognitive function level and MAP-2 protein levels; in addition, over time, the cognitive function level and MAP-2 protein expression level in the intervention group and 5% IH group showed a gradual reduction, while ERK expression level was not significantly different within the intervention group, suggesting that hypoxia-induced cognitive changes may not only related with the activation of ERK signaling pathway in rat hippocampal CA1 region and decreased MAP-2 expression, but also be affected by other mechanisms of action. There are few studies on the mechanism of ERK pathway regulating MAP-2 expression currently at home and abroad; in the chronic intermittent hypoxia, the role of ERK pathway in mediating the degradation of MAP-2 and cognitive dysfunction still needs to be further studied by more targeted research, so as to finally reveal the mechanism of cognitive dysfunction in patients with OSAHS.

Disclosure of conflict of interest

None.

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References

[1] McNicholas WT and Bonsigore MR. Management Committee of EU COST ACTION B26. Sleep apnoea as an independent risk factor for cardiovascular disease: current evidence, basic mechanisms and research priorities. Eur Respir J 2007; 29: 156-178.

[2] Houser CR, Huang CS and Peng Z. Dynamic seizure-related changes in extracellular signal-regulated kinase activation in a mouse model of temporal lobe epilepsy. Neuroscience 2008; 156: 222-237.

[3] Jin YS and Yuan B. Microtubule associated protein-2 expression changes of hippocampal infarction rats learning and memory abilities impact study. Zhong Guo Shi Yan Zhen Duan Xue 2014; 18: 538-541.

[4] Zhang PP, Han XQ, Wang HY, Yu JT, Li L, Wang LM and Guo XH. Intermittent hypoxia rats neuron specificity enolization of enzyme protein expression and its effects on learning and

memory function. Zhong Hua Xing Wei Yi Xue Yu Nao Ke Xue Za Zhi 2014; 23: 303-306.
[5] Wang HY, Zhao YN, Guo X, Yu CL and Li L. The cognitive function and depression status of patients with severe OSAHS on impact. He Bei Yi Yao 2011; 33: 3725-3726.
[6] Silingardi D, Angelucci A, De Pasquale R, Borsotti M, Squitieri G, Brambilla R, Putignano E, Pizzorusso T and Berardi N. ERK pathway activation bidirectionally affects visual recognition memory and synaptic plasticity in the perirhinal cortex. Front Behav Neurosci 2011; 5: 84.
[7] Kong QX, Liang RQ, Gao JY, Sun R, Li L, Zhu X and Xia M. Epilepsy resistance to promote intellectual epilepsy drugs on cognitive dysfunction rats hippocampus extracellular signal regulating kinase 2 and neural cell adhesion molecule 1 expression. Zhong Hua Xing Wei Yi Xue Yu Nao Ke Xue Za Zhi 2013; 22: 696-699.
[8] Zhuang S and Schnellmann RG. A death-promoting role for extracellular signal-regulated kinase. J Pharm acol Exp Ther 2006; 319: 991-997.
[9] Park ER, Eblen ST and Catling AD. MEK1 activation by PAK: A novel mechanism. Cell Signal 2007; 19: 1488-1496.
[10] Marchetti S, Gimond C, Chambard JC, Touboul T, Roux D, Pouysségur J and Pagès G. Extracellular signal-regulated kinases phosphorylate mitogen activated protein kinase phosphatase 3/DUSP6 at serines 159 and 197, two sites critical for its proteasomal degradation. Mol Cell Biol 2005; 25: 854-864.
[11] Wu X, Yang L, Wang BJ, Zhang GH, Zhen B, Sun HJ, Wang M and He BL. The MAP-2 after the injury rats express change regularity study time. Zhong Guo Fa Yi Xue Za Zhi 2011; 26: 267-272.
[12] Kang JQ, Chong ZZ and Maiese K. Critical role for Akt1 in the modulation of apoptotic phosphatidylserine exposure and microglial activation. Mol Pharmacol 2003; 64: 557-569.
[13] An GH, Chen XW, Wang J, Liu HT and Ma Q. Chronic unpredictable on the psychological stress rats cognitive function and the hippocampus microtubule associated protein-2 expression. Jie Fang Jun Yu Fang Yi Xue Za Zhi 2012; 30: 239-242.
[14] Ma ZJ, Niu HY, Yi XN, Li MB and Wen XD. Vascular dementia model of rat hippocampal CA3 area of microtubule associated protein-2 expression and its significance. Xian Dai Sheng Wu Yi Xue Jin Zhan 2009; 9: 35-38.