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CIH-induced neurocognitive impairments are associated with hippocampal Ca^{2+} overload, apoptosis, and dephosphorylation of ERK1/2 and CREB that are mediated by overactivation of NMDARs

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ABSTRACT

Chronic intermittent hypoxia (CIH) is commonly seen in patients with obstructive sleep apnea, and has been hypothesized to underlie the neurocognitive dysfunction in these patients. However, its cellular and molecular mechanisms remain to be defined. The present study aimed to investigate, in a mouse CIH model, the role of NMDA receptor (NMDAR) activation in mediating the CIH-induced neurocognitive impairments, caspase expression and dysregulated Ca^{2+} signaling pathways in hippocampus. Male ICR mice (n=45) were exposed to CIH (8 h/day) or room air (control) for 4 weeks. After 4-week treatment, neurobehavioral assessments were performed by Morris water maze test, hippocampal $[Ca^{2+}]$i was evaluated by flow cytometry; and protein expressions of caspase-3, caspase-9, PARP, p-ERK1/2 and p-CREB in hippocampus were measured by Western blotting. Our results showed that, compared to control animals, 4-week exposure to CIH produced significant spatial learning and memory impairments in CIH mice. Increased caspase expression in hippocampus was observed in CIH mice associated with significant elevation of $[Ca^{2+}]$i and dephosphorylation of ERK and CREB expression. When the NMDAR antagonist memantine was administered by intraperitoneal injection prior to daily exposure to CIH, at a sub-therapeutic dose of 5 mg/kg/day not shown to impact the

Abbreviations: OSA, obstructive sleep apnea; CIH, chronic intermittent hypoxia; NMDAR, *N*-methyl-D-aspartate receptor; $[Ca^{2+}]$i, intracellular calcium concentration; ERK1/2, extracellular-signal-regulated kinase 1/2; CREB, cyclic adenosine monophosphate response element binding protein; p-ERK 1/2, phosphorylated extracellular-signal-regulated kinase 1/2; p-CREB, cyclic adenosine monophosphate response element binding protein

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neurobehavioral performance in control animals, the neurocognitive impairments as well as the neurobiochemical changes were abolished or normalized in the CIH mice. Our study suggests that overactivation of NMDARs and the Ca^{2+} overload-dependent ERK/CREB dysregulation is one of the important mechanisms in mediating the CIH-induced neurocognitive impairments.

1. Introduction

Obstructive sleep apnea (OSA) is a common type of sleep disorder in which the upper airway collapses repeatedly during sleep, resulting in chronic intermittent hypoxia (CIH) and associated sleep fragmentation (Young et al., 1993). In addition to cardiovascular and metabolic morbidities, OSA can cause a broad range of neurocognitive deficits from attention and vigilance impairment to memory and executive dysfunctions (Chen et al., 2011; Jackson et al., 2011; Kheirandish-Gozal et al., 2013), which can profoundly impact the individual work and quality of life. In rodents, exposure to CIH during sleep that produced the similar nocturnal hypoxia/re-oxygenation cycles in OSA have been shown to manifest not only metabolic disorders (Messenger et al., 2013) but also certain features of neurocognitive dysfunction seen in OSA patients, such as learning and memory deficits and impaired vigilance (Gozal et al., 2001; Row et al., 2002; Nair et al., 2011). Multiple pathophysiological processes have been proposed to contribute to the cognition dysfunction associated with CIH, and these include increased oxidative stress (Row et al., 2003; Xu et al., 2004) and inflammation (Ryan et al., 2007), altered gene regulation (Kheirandish et al., 2005a), and dysregulation of the cellular and molecular substrates of synaptic plasticity (Xie et al., 2010). However, the exact cellular and molecular mechanisms underlying the CIH-induced neurocognitive impairments remain to be defined.

Glutamatergic neurotransmission plays a vital role in mediating synaptic plasticity and receptor function in the regulation of learning and memory. *N*-methyl-D-aspartate receptors (NMDARs) are cation channels gated by the neurotransmitter glutamate and the essential regulators of neuronal development, synaptic transmission and plasticity (Bliss and Collingridge, 1993; Aamodt and Constantine-Paton, 1999). In neurodegenerative diseases such as Huntington's disease and Alzheimer's disease (AD), ischemic stroke and hypoxic stress, excessive release of glutamate results in an overactivation of NMDARs and the downstream effectors that are implicated in neuronal damage and cell death. This process is known as synaptic excitotoxicity (Olney et al., 1971; Huo et al., 2014). Choi (1987), Choi et al. (1987, 1988) has reported that the overactivation of NMDARs and subsequent neuronal Ca^{2+} overload trigger a cascade of downstream events leading to excitotoxicity. The Ca^{2+} dependent signaling pathways are the key component of the molecular and cellular mechanisms underlying learning and memory, and these include Ca^{2+}/calmodulin-dependent protein (CaM) kinase, Ras-extracellular-signal regulated kinase (ERK) 1/2 signaling pathways and the transcription factor cyclic-AMP response element binding protein (CREB). Dysregulation of neuronal Ca^{2+} homeostasis resulting in abnormal Ca^{2+}-dependent signaling transduction, mitochondrial dysfunction and programmed cell death has been hypothesized to contribute to the neurocognitive impairments in a number of CNS disease states (Kalia et al., 2008; Wroge et al., 2012).

Recently, Huo and his colleagues reported that rat exposed to chronic intermittent hypoxia-hypercapnia (CIHH) displayed learning and memory deficits, which may be caused by excitotoxicity with increased NR2B subunit expression and dysregulated downstream signaling cascade. CIHH is an animal model of chronic obstructive pulmonary disease (COPD), and it differs from CIH in that the CO_2 level in CIHH model is much higher compared to CIH model (Huo et al., 2014). Hence, the role of synaptic excitotoxicity in CIH-induced neurocognitive impairments warrants further evaluations.

In the present research, we aimed to examine for the first time the role of NMDARs overactivation and the abnormalities of Ca^{2+}-dependent signaling pathways in neurocognitive dysfunction observed in a mouse model of CIH. The effect of 4-week CIH (8 h/day) on the neurobehavioral performance was assessed by the Morris water maze test, as well as the associated changes in intracellular calcium concentration ($[Ca^{2+}]i$), downstream protein expression of ERK1/2, CREB, and apoptosis in the hippocampus. In addition, we examined the effect of memantine, a noncompetitive NMDAR antagonist, on the CIH-induced neurocognitive deficits and dysregulation of $[Ca^{2+}]i$ and ERK/CREB protein expression to determine the importance of NMDARs overactivation in mediating these changes and evaluate the underlying mechanisms.

2. Results

2.1. *Effect of CIH and memantine pretreatment on MWM test*

In the invention phase of the present study as described in Section 4, a total of 45 male ICR mice (4-week old, 15 animals per group) exposed to CIH or room air (RA) for 4 weeks was tested for learning and memory ability using the Morris water maze (MWM) test. In the place navigation test, CIH+VEH group showed significantly longer escape latencies (Day 3: 40.91 ± 2.95 s vs. 31.55 ± 2.78 s, $p<0.05$; Day 4: 37.49 ± 3.20 s vs. 22.48 ± 2.77 s, $p<0.01$) and longer distance travelled (Day 3: 1045.07 ± 84.40 cm vs. 751.26 ± 67.87 cm, $p<0.05$; Day 4: 922.39 ± 85.46 cm vs. 579.65 ± 72.70 cm, $p<0.05$) compared with RA group. Pre-treatment with memantine (CIH+MEM)