Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Research Report

Chronic intermittent hypoxic preconditioning suppresses pilocarpine-induced seizures and associated hippocampal neurodegeneration

Jun-li Zhen[a], *Wei-ping Wang*[a,*], *Jing-jing Zhou*[b], *Zhen-zhen Qu*[a], *Hai-bo Fang*[a], *Ran-ran Zhao*[a], *Yan Lu*[a], *Hong-chao Wang*[a], *Hong-min Zang*[a]

[a]*Key Laboratory of Neurology of Hebei Province, The Second Hospital of Hebei Medical University, Shijiazhuang 050000, Hebei, China*

[b]*Department of Physiology, Hebei Medical University, 361 Zhongshan East Road, Shijiazhuang 050017, Hebei, China*

ARTICLE INFO

Article history:
Accepted 20 March 2014
Available online 27 March 2014

Keywords:
Hypoxia
Pilocarpine
Epilepsy
Spontaneous recurrent seizures

ABSTRACT

Mild brief hypoxia can protect against neuronal damage induced by epileptic seizures, at least in part by inhibiting apoptosis. Further elucidation of the antiepileptic mechanisms and optimization of the conditioning protocols are required before this strategy can be considered for clinical intervention. In this study, we compared the effects of different hypoxic preconditioning protocols on spontaneous recurrent seizures (SRS), intracellular free calcium concentration ($[Ca^{2+}]i$), and apoptosis rate following pilocarpine-induced status epilepticus (SE). Male Sprague Dawley rats were subjected to either chronic intermittent hypobaric hypoxia (CIHH) or chronic intermittent normobaric hypoxia (CINH) (both for 6 h/day × 28 consecutive days) prior to pilocarpine-induced SE. The possible anticonvulsant and neuroprotective effects of CIHH and CINH were compared by video monitoring of behavioral seizure activity (frequency, delay), Nissl staining and Fluoro-Jade B (FJB) staining to examine changes in the morphology of hippocampal pyramidal neurons, and flow cytometry to detect the quantification of $[Ca^{2+}]i$ and cell apoptosis. Both hypoxic preconditioning protocols reduced the frequency and severity of SRS, suppressed post-ictal $[Ca^{2+}]i$ elevations, and inhibited neuronal apoptosis in the rat hippocampus compared to pilocarpine alone, but CIHH was more effective than CINH. Thus, mild hypoxic pretreatment, particularly when delivered as CIHH, may be a novel strategy for the clinical prevention and treatment of epilepsy.

Abbreviations: SE, status epilepticus; SRS, spontaneous recurrent seizures; $[Ca^{2+}]i$, intracellular free calcium concentration; CIHH, chronic intermittent hypobaric hypoxia; CINH, chronic intermittent normobaric hypoxia

*Corresponding author. Fax: +86 311 66002915.
E-mail addresses: WWP_ZJL_456@163.com, zhenjunli666@163.com (W.-p. Wang).

http://dx.doi.org/10.1016/j.brainres.2014.03.032
0006-8993/

1. Introduction

Epilepsy is a chronic and in many cases intractable or fatal brain disease due to spreading neuronal hyperexcitability (Reddy and Kuruba, 2013). Even if treatable, sporadic seizures and treatment side effects can greatly reduce activities of daily life.

The progressive functional deficits observed in epilepsy patients likely arise from apoptosis and necrotic death of vulnerable neurons, particularly in areas such as the hippocampus. Epilepsy-induced neuronal death may also increase the likelihood of recurrence, thereby compounding disability (Moran et al., 2013; Yis et al., 2013). Sustained elevation of the neuronal intracellular calcium concentration ([Ca^{2+}]i) or "calcium overload" during and following seizures can trigger apoptosis by promoting release of pro-apoptotic factors such as cytochrome c from mitochondria, resulting in activation of effector caspases pathways (Yu Canzoniero and Choi, 2001). Unfortunately, currently available antiseizure drugs do not directly prevent these pathogenic processes; hence, more effective supplemental or alternative treatments are required to improve clinical outcome.

Acute hypoxia, deficits in a variety of injury due to inadequate oxygen supply or utilization, is also a leading cause of neurodegeneration and neurological disability (Shukitt-Hale Banderet and Lieberman, 1998). However, mild transient hypoxia preconditioning has been shown repeatedly to enhance neural resistance to subsequent insults. This preconditioning effect is mediated by the activation of numerous genes involved in adaptation and neuroprotection, including hypoxia-inducible transcription factors that in turn activate genes associated with erythropoiesis, angiogenesis, glucose metabolism, cell proliferation, anti-apoptotic pathways, and the antioxidant response (Bernaudin et al., 2002; Jones and Bergeron, 2001; Rodriguez et al., 1999; Rybnikova et al., 2005a, 2005b). Indeed, hypoxic preconditioning has been demonstrated to suppress cell damage and functional deficits in a variety injury models, such as oxidative damage from renal ischemia-reperfusion (Yang et al., 2009) and apoptosis of cardiac myocytes under hypoxia (Dong et al., 2003). Moreover, preconditions preserves neurological function and elevates neuronal tolerance to ischemia or ischemic-reperfusion injury (Ara et al., 2011; Ding et al., 2002; Rybnikova et al., 2005a, 2005b; Samoilov et al., 2003). The phenomenon of hypoxic preconditioning has attracted the interest of both basic researchers interested in mechanisms of endogenous cytoprotection and clinical researchers attempting to translate these finding in beneficial treatments (Casas et al., 2000). However, many molecular aspects of these neuroprotective mechanisms remain obscure and longer term outcomes are not yet known. In addition to continued study of the molecular pathways of neuroprotection, it is still necessary to explore optimal induction protocols that may provide a foundation for the safe clinical application of hypoxic preconditioning.

Pilocarpine is a cholinergic agonist used to induce seizures that resemble those observed in human temporal lobe epilepsy, particularly with regard to delayed neuronal damage (Cavalheiro et al., 1991) and continuity of spontaneous recurrent seizures (SRS) (Li et al., 2013). Several previous studies concluded that normobaric hypoxic preconditioning is superior to normobaric hypoxic postconditioning for protection against neuronal apoptosis in epilepsy models (Amano et al., 1990; Yang et al., 2013). However, few studies have systematically compared the protective efficacy of different hypoxic preconditioning protocols against SRS, neuronal [Ca^{2+}]i overload, and apoptosis after status epilepsy (SE).

The aim of present study was to compare preconditioning by chronic intermittent hypobaric hypoxia (CIHH) to chronic intermittent normobaric hypoxic (CINH) for suppression of pilocarpine-induced SRS and both [Ca^{2+}]i elevation and apoptosis of rat hippocampal neurons. Optimization of preconditioning protocols is a necessary first step for eventual clinical use to prevent and treat epilepsy.

2. Results

2.1. CIHH-Pre and CINH-Pre reduced mortality and the frequency of spontaneous recurring seizures (SRS) in pilocarpine-treated rats

Pilocarpine injection caused significant mortality in naïve Sprague Dawley rats, while both CIHH-Pre and CINH-Pre significantly reduced mortality following subsequent pilocarpine injection (pilocarpine only group: 47.1%, CIHH-Pre: 16.7%, CINH-Pre: 20.8%). No fatalities were observed in the control group, indicating that vehicle, lithium-atropine sulfate, and chloral hydrate i.p. injections had no effect on the health of these rats. Pilocarpine-induced SE was followed 14±2 days later by SRS. The number, severity, and duration of these spontaneous seizures were recorded from 15 days to one month after SE. As shown in Fig. 1, there was a significant decrease in the number, mean severity, and duration of seizures in both CIHH-treated and CINH-treated SE rats compared to those treated by pilocarpine alone ($P<0.01$ for

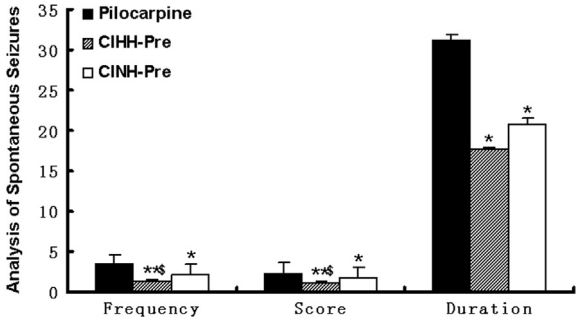

Fig. 1 – Fifteen days after SE induction, seizure number, severity, and duration were recorded, with video monitoring performed 6 h per day over a 1-month period among the pilocarpine, CIHH-Pre, and CINH-Pre groups ($n=5$/group). Seizure frequency, severity, and duration were reduced in both CIHH-treated and CINH-treated SE rats compared to those treated with pilocarpine alone. Seizure severity was scored by a modified Racine's scales. The CIHH-Pre protocol induced a greater reduction in both seizure frequency and severity compared to the CINH-Pre group. $^{*}P<0.05$ and $^{**}P<0.01$ compared to the pilocarpine group; $^{\$}P<0.05$ compared to the CINH-Pre group.