BRAIN RESEARCH 1538 (2013) 93–103

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Neuroprotective effects of prior exposure to enriched environment on cerebral ischemia/reperfusion injury in rats: The possible molecular mechanism

Kewei Yu[a,b], *Yi Wu*[a,b,c,d,*], *Yongshan Hu*[a,b,d], *Qi Zhang*[a,b], *Hongyu Xie*[a,b], *Gang Liu*[a,c], *Yao Chen*[c], *Zhenzhen Guo*[a,b], *Jie Jia*[a,b,d,*]

[a]*Department of Rehabilitation, Huashan Hospital, Fudan University, Shanghai, 200040, China*
[b]*State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai, 200032, China*
[c]*The Yonghe Branch of Huashan Hospital, Fudan University, Shanghai 200436, China*
[d]*Department of Sports Medicine and Rehabilitation, Medical College of Fudan University, Shanghai 200032, China*

ARTICLE INFO

Article history:
Accepted 23 September 2013
Available online 29 September 2013

Keywords:
Enriched Environment
Cerebral ischemia
ERK1/2
iNOS
nNOS
MDA

ABSTRACT

Increasing evidence shows that exposure to an enriched environment (EE) after cerebral ischemia/reperfusion injury is neuroprotective in animal models. Recent studies have demonstrated that animals housed in an enriched environment condition after an experimental stroke obtained a better functional outcome than those housed in a standard condition. However, little is known about the underlying mechanisms of neuroprotective effects of enriched environment exposure prior to injury. The current study examined the neuroprotective effects of prior enriched environment exposure after transient middle cerebral artery occlusion (MCAO) in rats. Male Sprague Dawley (SD) rats, weighing 55–65 g at the beginning of the experiment, were randomly assigned to a pre-ischemic enriched environment (PIEE) or pre-ischemic standard condition (PISC) group for 1 month. They were weighed on days1, 7, 18, and 28, and their locomotor activity was tracked during the period between 9:00 am and 3:00 pm daily. After 1 month, ischemia was induced by occluding the middle cerebral artery for 90 min, followed by reperfusion. After approximately 24 h of the operation, functional outcomes were assessed using the beam-walking test and a neurological evaluation scale in all rats. We measured the expression of extracellular signal regulated protein kinases1/2 (ERK1/2) by western blotting and gene expression levels of neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthasen (iNOS) by Real-Time PCR in the cortical area affected by ischemia. Finally, we measured the level of malondialdehyde (MDA) content, which is a biomarker of oxidative stress. The results showed that rats in the PIEE group had lighter weight than those in the PISC group. The functional outcomes of rats in the PIEE group were better than those in the

Abbreviations: PIEE, pre-ischemic enriched environment; PISC, pre-ischemic standard condition; BIT, brain ischemic tolerance; MDA, malondialdehyde; nNOs, neuronal nitric oxide syntheses; iNOS, inducible nitric oxide syntheses; ERK1/2, extracellular signal regulated protein kinases1/2; p-ERK1/2, phosphorylated extracellular signal regulated protein kinases1/2; tMCAO, transient middle cerebral artery occlusion.

*Corresponding author at: Corresponding authors at: Department of Rehabilitation Medicine, Huashan Hospital, Fudan University, WuLuMuQi Middle Road 12, Shanghai, 200040, China. Fax: +86 21 52887820.
E-mail addresses: wuyi@126.com (Y. Wu), jiajie@163.com (J. Jia).

0006-8993/$ - see front matter
http://dx.doi.org/10.1016/j.brainres.2013.09.036

PISC group, and substances associated with inflammation, such as MDA, nNOS, iNOS, and phospho-ERK1/2, were lower in the PIEE group compared with the PISC group. These results indicate that enriched environment may provide neuroprotection via ischemic preconditioning and enhance resilience to cerebral ischemia.

1. Introduction

Ischemic stroke is considered as one of the leading causes of death and disability around the world that results in a significant societal burden (Moseley et al., 2003; Murray and Lopez, 1997).Yet there are few treatment options available for most types of strokes. Therefore, prevention is crucial for those at high-risk of strokes. The ability of the brain to develop tolerance to subsequent ischemic injury after moderate hypoxia is known as the brain ischemic tolerance (BIT) phenomenon (Kitagawa et al., 1990). It is important to find an appropriate and effective method of promoting resistance to ischemic stroke that would alleviate the symptoms of strokes and their consequences. Several studies have demonstrated that certain pretreatments such as pre-exercise using treadmill training can induce resistance to cerebral ischemia and reduce the risk of a stroke (Guo et al., 2008; Zhang et al., 2010).

An enriched environment has been demonstrated to be neuroprotective in brain-damaged animals (Buchhold et al., 2007 Pereira et al., 2008), and beneficial effects of enriched environment were also reported in animals that sustained either focal or global ischemic injury (Biernaskie and Corbett, 2001). Our latest research shows that PIEE may induce BIT through increasing physical activity (Xie et al., 2013).

One of the key elements in the pathophysiology and outcomes of cerebral ischemia is inflammation (Emsley and Tyrrell, 2002). The neuronal and inducible isoforms of NOS (nNOS, iNOS) and malondialdehyde (MDA) are involved in inflammation and the oxidation reaction process. nNOS and iNOS enhance the production of peroxynitrite, a free radical involved in lipid peroxidation, suppress mitochondrial respiratory enzymes, and damage DNA (Awooda et al., 2013). Furthermore, some reports have demonstrated that the inhibition of nitric oxide synthase (NOS) activity diminishes acute ischemic brain damage (Mohammadi et al., 2012; Ding-Zhou et al., 2002). MDA is one of the biomarkers of oxidative stress, and it is associated with the harmful consequences of cerebral ischemia. However, whether prior exposure to enriched environment could reduce the release of nNOS, iNOS, and MDA caused by cerebral ischemia is still unknown.

The extracellular signal regulated protein kinases 1/2 (ERK1/2) pathway has been shown to play a key role in the neuroprotective effect of pre-ischemic exercise (Liebelt et al., 2010), and our group recently reported that pre-ischemic treadmill training might induce neuroprotection by inhibiting phospho-ERK1/2 over-activation (Zhang et al., 2010). A growing number of studies have suggested that the ERK pathway actually exacerbates neurological damage, increases oxidative stress-related death, and promotes inflammation (Sawe et al., 2008). Furthermore, inhibition of the ERK1/2 pathway has been shown to reduce brain damage after ischemia (Namura et al., 2001; Noshita et al., 2002). Therefore, we hypothesized that one of the underlying mechanisms of enriched environment in protection of cerebral ischemia may be through the suppression of ischemic inflammation in the brain of the ischemic rats.

This study was designed to explore whether prior exposure to enriched environment provided protection to rats with subsequent cerebral ischemia, and we investigated the phospho-ERK1/2, nNOS, iNOS, and MDA which are related to inflammation at 24 h after tMCAO.

2. Results

2.1. *Body weight*

During the experiment, we measured all the rats' body weights on day 1, 7, 18, and 28. As shown in Fig. 1A, rats in the PIEE group weighed less than those in the PISC group at day 7, 18, and 28.

2.2. *Enriched environment increased locomotor activity*

We quantified the locomotor activity of individual rats from the PIEE and PISC groups by using an automatic tracking system during the 1 month of observation between 9:00 am and 3:00 pm daily. As shown in Fig. 1, PIEE had a higher level of locomotor activity during the 28-day observation period compared to PISC. There was a significant difference between the groups in the level of distance moved (Fig. 1B) and the duration of being mobile (Fig. 1C). These results demonstrate that an enriched environment improves voluntary activity in rats.

2.3. *Neurological status*

The neurological status was assessed 24 h after tMCAO using a 7-point scale. Sham-operated rats had no neurological symptoms, as indicated by neurological status scores of zero. As shown in Fig. 2A, the rats with PIEE had better neurological status scores compared to those with PISC. These results indicate that PIEE reduces neurological dysfunction following tMCAO.

2.4. *Beam-walk task*

A beam-walk task was used to assess the deficits in coordination and integration of motor movement in the rats after ischemia. As shown in Fig. 2B, the rats with PIEE had better scores in the beam-walking test compared to those with PISC.