Chapter 27
Effects of Enriched Environment on Hippocampal Neuronal Cell Death and Neurogenesis in Rat Global Ischemia

Tomokazu Kato, Takashi Eriguchi, Norio Fujiwara, Yoshihiro Murata, Atsuo Yoshino, Kaoru Sakatani, and Yoichi Katayama

Abstract Enriched environments reportedly show neuroprotective effects. Here, we evaluated the effect of an enriched environment prior to cerebral ischemia on neuronal cell death and neurogenesis in rats. Male SD rats were housed under standard conditions (SC) or in an enriched environment (EE), then subjected to global ischemia. The Y-maze test and novel object cognition test were used to evaluate cognitive function before and after ischemia. At 7 days post-ischemia, we evaluated hippocampal neuronal cell death with Fluoro-Jade B staining and neurogenesis with BrdU staining. Phosphorylated cAMP response element-binding protein (phospho-CREB) was also evaluated immunohistochemically. The EE + ischemia group showed a significant decrease of cell death post-ischemia compared with the SC + ischemia group. There was no difference in neurogenesis post-ischemia between SC + ischemia and EE + ischemia. The EE + ischemia group showed a significant increase of performance before and after ischemia compared with the SC + ischemia group. Phospho-CREB-positive cells were significantly increased post-ischemia in EE + ischemia

T. Kato • T. Eriguchi • N. Fujiwara • Y. Murata • A. Yoshino • Y. Katayama
Division of Neurosurgery, Department of Neurological Surgery,
Nihon University School of Medicine, 30-1 Oyaguchi-Kamimachi,
Itabashi-ku, Tokyo 173-8610, Japan
e-mail: tom_kato@rk9.so-net.ne.jp

K. Sakatani (✉)
Division of Neurosurgery, Department of Neurological Surgery, Nihon University School of Medicine, 30-1 Oyaguchi-Kamimachi, Itabashi-ku, Tokyo 173-8610, Japan

Department of Electrical and Electronics Engineering, Nihon University College of Engineering, NEWCAT Institute, Fukushima, Japan
e-mail: sakatani.kaoru@nihon-u.ac.jp

H.M. Swartz et al. (eds.), *Oxygen Transport to Tissue XXXVI*, Advances in Experimental Medicine and Biology 812, DOI 10.1007/978-1-4939-0620-8_27,

compared with SC + ischemia. EE suppressed hippocampal cell death due to global ischemia. Additionally, enhancement of cognitive function before and after ischemia and prevention of cognitive impairment associated with ischemia were observed compared with the controls (rats housed in SC without ischemia). The CREB pathway may play an important role in protection of cognitive ability.

Keywords Enriched environment • Cerebral ischemia • Neurogenesis • Neuronal cell death • Hippocampus

1 Introduction

Vulnerability of the hippocampus to cerebral ischemia has been established in numerous studies. Selective and delayed neuronal cell death after global ischemia occurs in the subgranular zone (SGZ) in rats [1]. It was also reported that neurogenesis proceeds constantly in the subventricular zone (SVZ) and SGZ [2]. Neurogenesis in SVZ and SGZ is deeply involved in memory, learning and mood disorders. In rat SGZ, neurogenesis is enhanced by learning [3] and neuronal damage, such as seizure and ischemic insult, and reduced by post-ischemic stress [4]. Thus, the environment influences hippocampal cell death and neurogenesis due to cerebral ischemia. We previously reported that a stress environment before cerebral ischemia increases neuronal cell death and impairs neurogenesis [5]. Effects of an enriched environment (EE) have been reported in some studies of cerebral ischemia. For example, EE after ischemia increases neuronal cell death [6] and enhances neurogenesis in rat hippocampus [7]. Here, we evaluated the influence of EE prior to cerebral ischemia on post-ischemic neuronal cell death in rats.

2 Methods

All experiments were performed following an institutionally approved protocol in accordance with the Nihon University School of Medicine Guide for the Care and Use of Laboratory Animals.

Twenty-four Sprague-Dawley male rats (250–300 g) were anesthetized with isoflurane (1–1.2 %) in 30 % oxygen/70 % nitrous oxide. Their temperature was maintained at 37 °C with a heating pad. Femoral arteries were cannulated to monitor arterial blood pressure, pH and blood gases. Rats were assigned into the following groups: controls housed under standard conditions (SC) (n = 6); EE (housed in an EE without ischemia) (n = 6); SC + ischemia (n = 6); ischemia following EE (EE + ischemia) (n = 6). Global ischemia was induced by bilateral carotid arterial occlusion combined with hypotension for 10 min [8]. The EE consisted of a

stainless steel cage (800 × 400 × 610 mm) containing wooden logs, tubes and shelves for climbing, and a running wheel. These rats were housed four per cage. On the other hand, in the SC group, rats were housed in pairs in standard laboratory cages (255 × 220 × 150 mm). All groups were housed SC or EE for 6 weeks.

Cognitive function was evaluated by means of the Y-maze test and a novel object recognition test (ORT) after 6 weeks SC or EE and after ischemia. Both tests have been used previously for cognitive assessment of rats [9–11]. Rats were euthanized at 7 days after ischemia. Reduction rates of Y-maze and ORT values were calculated as (pre-ischemia − post-ischemia)/pre-ischemia × 100 to assess differences between pre- and post-ischemia in the EE + ischemia group vs the corresponding differences in the SC + ischemia group. The brain was perfused with saline and 4 % paraformaldehyde, then 2 mm coronal sections were cut. Fluoro-Jade B staining and BrdU staining were used to evaluate hippocampal neuronal cell death and neurogenesis, respectively. Fluoro-Jade B-positive cells were counted in a 1 mm length of a horizontal section of the hippocampal CA1 area. BrdU-positive cells were counted in 1 mm^2 of hippocampal dentate gyrus. Staining of phosphorylated cAMP response element-binding protein (phospho-CREB) was also performed in the two groups subjected to ischemia, and positive cells were counted in the same manner as in the case of Fluoro-Jade B staining. Positively stained cells in each case were evaluated in a double-blind manner. Data were expressed as mean ± SD. The significance of differences in positive cell counts was assessed by means of ANOVA followed by Tukey-Kramer tests. Differences with $p \leq 0.05$ were considered significant.

3 Results

Hippocampal neuronal cell death in the two groups that had received global ischemia (SC + ischemia and EE + ischemia) was significantly increased compared with that in the two groups without ischemia (SC and EE) (Fig. 27.1a, b). Moreover, cell death in the EE + ischemia group was significantly decreased compared with that in the SC + ischemia group.

As for neurogenesis, BrdU-positive cells in the SC + ischemia and EE + ischemia groups were significantly increased compared with those in the SC and EE groups (Fig. 27.1c, d). However, no significant difference of BrdU-positive cells was observed between the SC + ischemia and EE + ischemia groups.

Phospho-CREB-positive cells in the SC + ischemia and EE + ischemia groups were also significantly increased compared with those in the SC and EE groups (Fig. 27.1e, f). Phospho-CREB-positive cells were significantly increased in EE + ischemia group compared with the SC + ischemia group.

Reduction rates in the two cognitive function tests in the EE + ischemia group were significantly lower than those in the SC + ischemia group (Fig. 27.2).In the case of ORT, there was no significant difference between before and after ischemia in the EE + ischemia group.

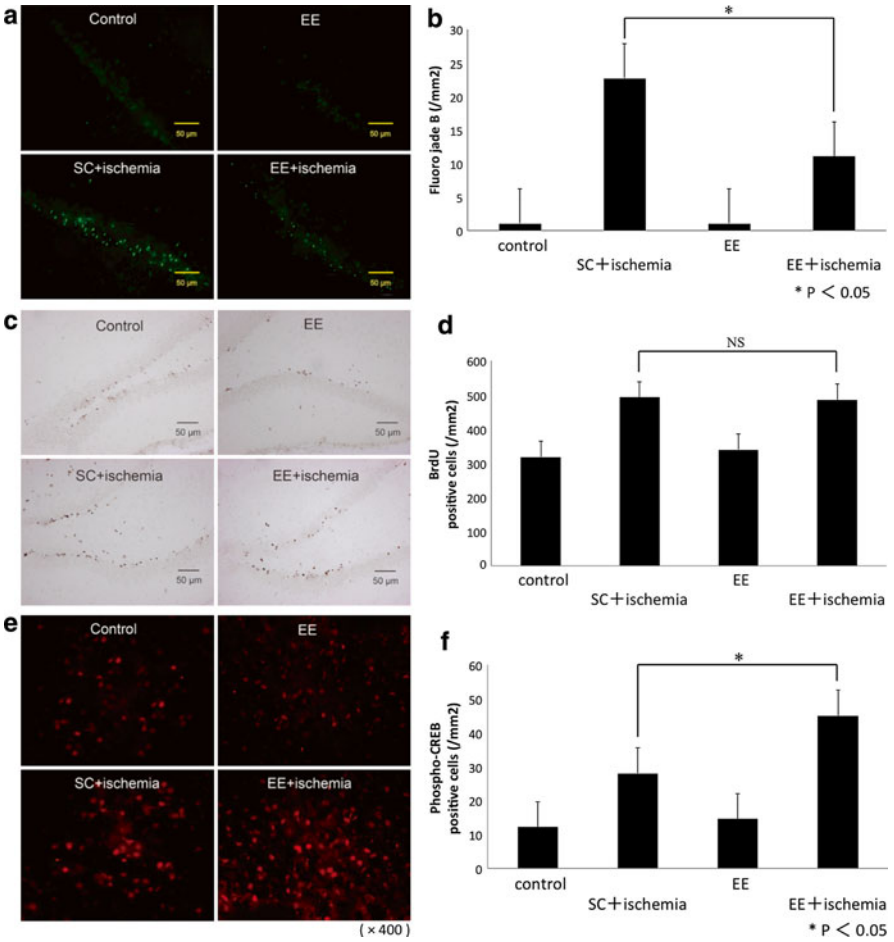

Fig. 27.1 Evaluation of neuronal cell death by Fluoro-Jade B staining (**a**), (**b**). (**a**) Hippocampal neuronal cell death in the EE + ischemia group was significantly decreased compared with that in the SC + ischemia group. (**b**) The results of statistical analysis of cell counts of Fluoro-Jade B-stained cells. Evaluation of neurogenesis by BrdU staining (**c**), (**d**). (**c**) BrdU-positive cells in the SC + ischemia and EE + ischemia groups were significantly increased compared with those in the SC and EE groups. There was no significant difference between the SC + ischemia and EE + ischemia groups. (**d**) The results of statistical analysis of cell counts of BrdU-stained cells. Evaluation of neurogenesis by phospho-CREB staining (**e**), (**f**). (**e**) Phospho-CREB-positive cells in the SC + ischemia and EE + ischemia groups were significantly increased compared with those in the SC and EE groups. Phospho-CREB-positive cells were significantly increased in the EE + ischemia group compared with the SC + ischemia group. (**f**) The results of statistical analysis of cell counts of phospho-CREB-stained cells

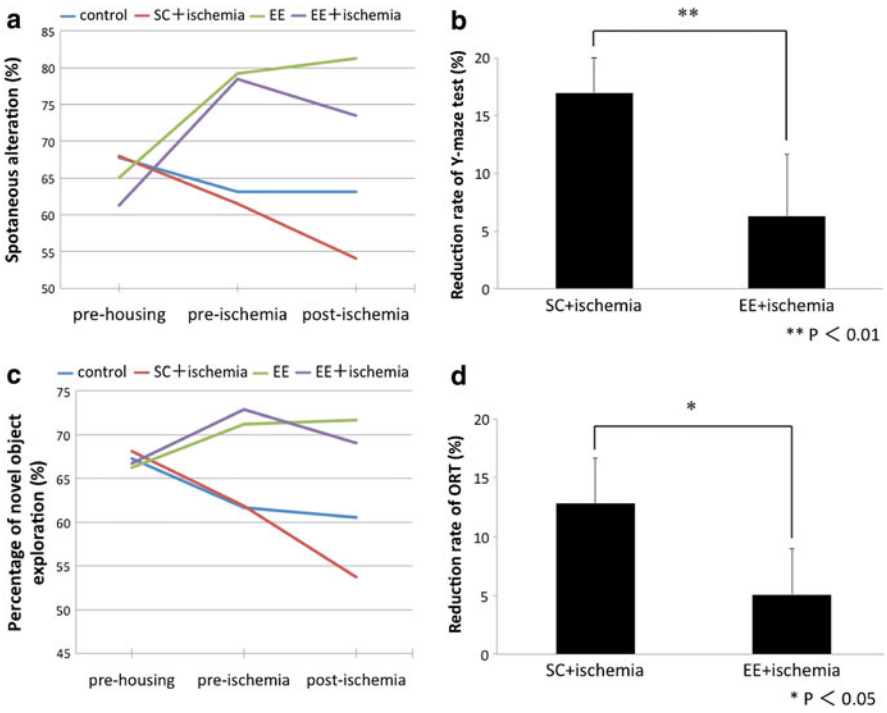

Fig. 27.2 Plots **a** (Y-maze test) and **c** (novel object recognition test (ORT)) show the results of cognitive function tests of all four groups. Significant enhancement of cognitive function before ischemia was observed in the EE + ischemia group compared with the SC + ischemia group in both tests ($p < 0.01$). Plots **b** (Y-maze test) and **d** (ORT) show the reduction rates of cognitive function. The reduction rates in the two cognitive function tests in the EE + ischemia group were significantly lower than those in the SC + ischemia group

4 Discussion

Exposure to EE after global cerebral ischemia leads to an increase in neuronal cell death and enhancement of neurogenesis in rat SGZ [6, 7]. However, little is known about whether EE prior to cerebral ischemia influences neuronal cell death and neurogenesis.

In the present study, exposure to EE prior to cerebral ischemia resulted in a decrease of neuronal cell death in the SGZ. This is a different response from that in rats exposed to EE after cerebral ischemia, because EE after cerebral ischemia increased neuronal cell death in rat hippocampus [6]. As regards neurogenesis, there was no difference between the EE + ischemia and SC + ischemia groups. The effect of EE prior to cerebral ischemia on neurogenesis may also be different from that of EE after ischemia. Expression of phospho-CREB in the SGZ was significantly increased in the EE + ischemia group compared with the SC + ischemia group.

It was reported that the CREB pathway is activated by ischemic insult [12], and the CREB pathway is related to cognitive function and anti-apoptotic effect through COX-2 and Bcl-2, respectively [13]. Our results suggest that activation of the CREB pathway may be related to enhancement of cognitive function before and after ischemia, prevention of cognitive impairment associated with ischemia and reduction of neuronal cell death associated with EE prior to ischemia. Additionally, the reduction of neuronal cell death itself may lead to the prevention of cognitive impairment associated with ischemia in the EE + ischemia group.

In conclusion, EE before global cerebral ischemia may reduce hippocampal cell death, enhance cognitive function before and after ischemia and prevent cognitive impairment associated with ischemia, compared with controls. The CREB pathway may play an important role in the neuronal cell death and enhancement and protection of cognitive ability associated with EE.

References

1. Kirino T (1982) Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res 239:57–69
2. Goldman SA, Nottebohm F (1983) Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain. Proc Natl Acad Sci U S A 80(8): 2390–2394
3. Brown ES et al (1999) Hippocampal remodeling and damage by corticosteroids: implications for mood disorders. Neuropsychopharmacology 21(4):474–484
4. Parent JM (2003) Injury-induced neurogenesis in the adult mammalian brain. Neuroscientist 9:261–272
5. Eriguchi T (2012) Influence of stress preconditioning on hippocampal neuronal cell death and neurogenesis in rat cerebral ischemia. Adv Exp Med Biol 737:57–61
6. Farrell R (2001) Environmental enrichment enhances recovery of function but exacerebates ischemic cell death. Neuroscience 107(4):585–592
7. Komitova M (2002) Effects of cortical ischemia and postischemic environmental enrichment on hippocampal cell genesis and differentiation in the adult rat. J Cereb Blood Flow Metab 22(7):852–860
8. Smith ML, Auer RN, Siesjo BK (1984) The density and distribution of ischemic brain injury in the rat following 2–10 min forebrain ischemia. Acta Neuropathol (Berl) 64:319–332
9. Nakamura K (2013) Effects of single and repeated electroconvulsive stimulation on hippocampal cell proliferation and spontaneous behaviors in the rat. Brain Res 23(1491):88–97
10. Gaskin S (2003) Retrograde and anterograde object recognition in rats with hippocampal lesions. Hippocampus 13(8):962–969
11. Komitova M (2005) Postischemic exercise attenuates whereas enriched environment has certain enhancing effects on lesion-induced subventricular zone activation in the adult rat. Eur J Neurosci 21(9):2397–2405
12. Kitadawa K (2012) CREB activation is a key player for ischemic tolerance in the brain. Rinsho Shinkeigaku 52(11):904–907
13. Watanabe T (2006) Cilostazol protects against brain white matter damage and cognitive impairment in a rat model of chronic cerebral hypoperfusion. Stroke 37(6):1539–1545