Chapter 9
Influence of Stress Preconditioning on Hippocampal Neuronal Cell Death and Neurogenesis in Rat Cerebral Ischemia

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1 Introduction

Numerous studies have demonstrated that the subgranular zone (SGZ) in dentate gyrus of the hippocampus is vulnerable in cerebral ischemia [1]. Selective and delayed neuronal cell death after global ischemia occurs in rat SGZ [2], and the delayed neuronal cell death is increased by stress after ischemia [3]. It was also reported that neurogenesis proceeds constantly in the subventricular zone (SVZ) and SGZ [4]. Neurogenesis in SVZ and SGZ is deeply involved in memory, learning, and mood disorders [5]. Neurogenesis in rat SGZ is enhanced by learning and neuronal damage, such as seizure and ischemic insult, and reduced by stress after ischemia [6].

In the present study, we evaluated the influence of stress prior to cerebral ischemia on neuronal cell death in rats.

2 Methods

All experiments were performed following an institutionally approved protocol in accordance with the guidelines of the Nihon University Laboratory Animal Research Committee.

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M. Wolf et al. (eds.), *Oxygen Transport to Tissue XXXIII*, Advances in Experimental Medicine and Biology 737, DOI 10.1007/978-1-4614-1566-4_9,

Twenty-four Sprague–Dawley male rats (250–300 g) were anesthetized with isoflurane (1–1.2%) in 30% oxygen/70% nitrous oxide. Temperature was maintained at 37°C with a heating pad. Femoral arteries were cannulated to monitor pressure, pH, and blood gases. Rats were assigned into the following groups: controls housed in normal cages ($n=6$); ischemia ($n=6$); stress ($n=6$); ischemia following stress ($n=6$).

Global ischemia was induced by means of bilateral carotid arterial occlusion for 10 min [7].

A forced swimming test was used to impose stress. Briefly, rats were obliged to swim from 2 days before induction of ischemia [8].

Rats were euthanized at 7 days after ischemia. The brain was perfused with saline and 4% paraformaldehyde, then 50 m 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 1 mm length of a horizontal section of hippocampal CA1 area. BrdU-positive cells were counted in 1 mm^2 of hippocampal dentate gyrus. Fluoro-Jade B-positive and BrdU-positive cells were evaluated in a double-blind manner.

Data are 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 $p<0.05$ were considered significant.

3 Results

Hippocampal neuronal cell death in the ischemia group, the stress group, and the ischemia following stress group was significantly increased compared with that in the control group (Fig. 9.1).

The ischemia following stress group showed a much greater increase of hippocampal neuronal cell death than the other groups.

As for neurogenesis, BrdU-positive cells in the ischemia group were significantly increased compared with the control and stress groups ($p<0.05$) (Fig. 9.2). In the ischemia following stress group, BrdU-positive cells were significantly decreased compared with the ischemia group ($p<0.05$). BrdU-positive cells in the stress group were decreased compared with the control. However, there was no significant difference in neurogenesis between the stress group and the control.

4 Discussion

Exposure to a stress condition after global cerebral ischemia leads to exacerbation of neuronal cell death in rat SGZ. However, little is known about whether stress prior to cerebral ischemia influences neuronal cell death and neurogenesis.

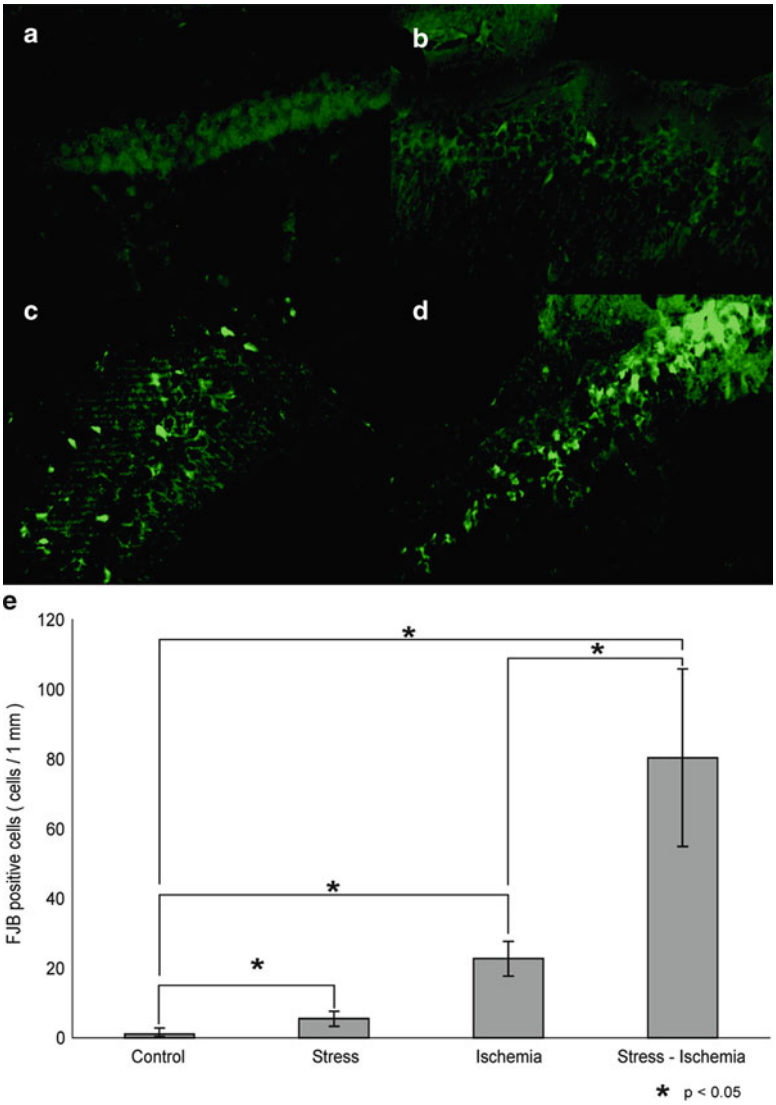

Fig. 9.1 Evaluation of neuronal cell death by Fluoro-Jade B staining. Hippocampal neuronal cell death in the ischemia group was significantly increased compared with that in the control group (**a**, **c**). The stress group also showed a significant increase of cell death compared with the control (**a**, **b**). The ischemia following stress group showed a much greater increase of hippocampal neuronal cell death than the other groups (**d**). The results of statistical analysis of cell counts of Fluoro-Jade B stained cells are summarized (**e**)

In the present study, exposure to a stress condition prior to cerebral ischemia resulted in an increase of neuronal cell death in SGZ. Previous studies have shown exacerbation of neuronal cell death in SGZ due to cardiac pulmonary arrest,

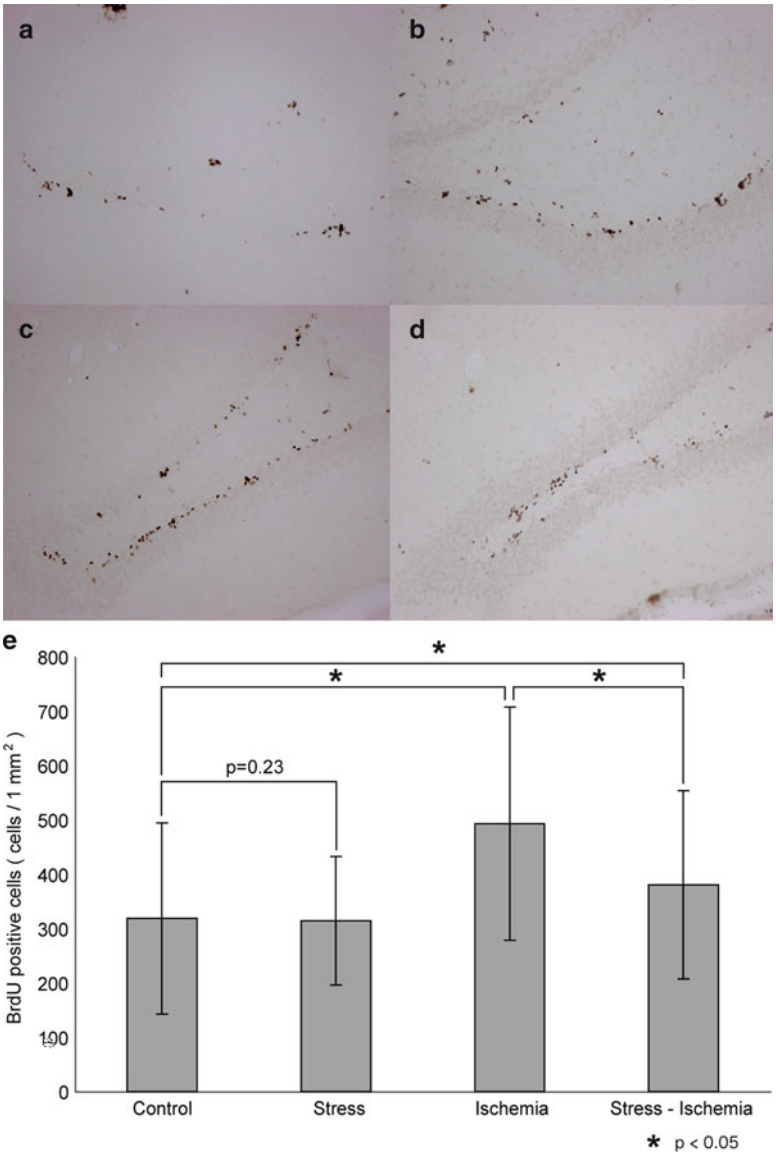

Fig. 9.2 Evaluation of neurogenesis by BrdU staining. BrdU-positive cells in the ischemia group were significantly increased compared with the control and stress groups. In the ischemia following stress group, BrdU-positive cells were significantly decreased compared with the ischemia group. There was no difference in neurogenesis between the control and stress groups. The results of statistical analysis of cell counts of BrdU-stained cells are summarized (**e**)

hypoglycemia, and so on [9]. Those stresses induce an increment of glucocorticoid, which exhibits neurotoxicity [10], and so may increase neuronal cell death after ischemia [11]. Glucocorticoid also decreases neurotropic factors, such as brain-derived

neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF), and therefore may inhibit neurogenesis as well as increasing neuronal cell death [12].

In conclusion, our results suggest that a stress environment before cerebral ischemia may increase neuronal cell death and impair neurogenesis. Thus, relief of stress may decrease damage and promote recovery following cerebral ischemia. These findings may also have implications for preventive medicine.

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