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Short Communication

Rip immunoreactivity significantly decreases in the stratum oriens of hippocampal CA1 region after transient forebrain ischemia in gerbils

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ABSTRACT

In the present study, we observed ischemia-related changes in Rip recognizing the promyelinating and myelinating oligodendrocytes in the hippocampus proper after 5 min of transient forebrain ischemia in gerbils. Rip immunoreactivity was significantly altered in the hippocampal CA1 region but not in the CA2/3 region after ischemic insult. In the sham-operated group, Rip immunoreactivity was shown in the cell bodies and processes of oligodendrocytes in all layers of the hippocampus proper. From 15 min to 2 days after ischemic insult, Rip immunoreactivity was similar to that of sham-operated group. Three days after ischemic insult, Rip-immunoreactive processes were tangled in the stratum oriens of the CA1 region, and Rip protein level decreased from this time after ischemia. Thereafter, Rip immunoreactivity was decreased time dependently in the CA1 region. Seven days after ischemic insult, Rip-immunoreactive processes were tangled and densely detected in the stratum oriens adjacent to the stratum pyramidale. In brief, these results indicate that the significant decrease of Rip immunoreactivity in processes in the stratum oriens of the hippocampal CA1 region occurs at late time after ischemia, and this decrease in Rip immunoreactivity may be associated with delayed neuronal death of CA1 pyramidal cells.

Ischemic injury to neurons is primarily due to the interruption of blood flow, lack of oxygenation, and subsequent re-oxygenation following brain ischemia/reperfusion (Frantseva et al., 2001; Hwang et al., 2005; Lee et al., 2004; Li et al., 2001; Petito et al., 1997; Sims and Anderson, 2002). The reperfusion induces intracellular oxidative damage caused by reactive oxygen species (ROS) (Numagami et al., 1996; Sturtz et al., 2001) because brain is highly susceptible to perioxidative damage because of its high lipid content and high oxygen requirement (Youdim et al., 1989).

Myelin formed by oligodendrocytes is crucial for the stabilization of axonal projections in the developing and adult mammalian brain. However, myelin components also act as a non-permissive and repellent substrate for outgrowing axons. Therefore, one major factor which accounts for the lack of axonal regeneration in the mature brain is myelin (Meier et al., 2004).

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The hippocampal CA1 pyramidal cells are selectively vulnerable to global ischemia, and neuronal death occurs in a delayed manner (Crain et al., 1988; Kirino, 1982; Petito et al., 1997). The threshold of global ischemia duration that induces neuronal death has been studied, but the relationship between ischemia duration and glial death in the hippocampal CA1 region has not been fully studied. Sugawara et al. (2002) examined glial viability and morphological changes in the CA1 region after cerebral global ischemia in rats. The number of astrocytes gradually declined after 5–10 min of ischemia, and viable astrocytes showed characteristic staged morphological reactions. Oligodendrocytes also showed morphological changes in their processes after ischemia, and microglia transformed into a reactive form at 5 days after ischemia (Sugawara et al., 2002).

The threshold of global ischemia duration that induces neuronal death has been studied, but the relationship between ischemia and glial changes in the hippocampus has not been fully studied. Hence, in the present study, we examined the temporal and spatial alterations of oligodendrocytes using Rip antibody, which does not detect astrocytes, microglia, or neurons (Friedman et al., 1989).

This study used the progeny of Mongolian gerbils (*Meriones unguiculatus*) obtained from the Experimental Animal Center, Hallym University, Chunchon, South Korea. The animals were housed in a temperature (23 °C)- and humidity (60%)-controlled room with a 12-h light/12-h dark cycle and provided with food and water ad libitum. Procedures involving animals and their care conformed to the institutional guidelines which are in compliance with current international laws and policies (*NIH Guide for the Care and Use of Laboratory Animals*, NIH Publication No. 85-23, 1985, revised 1996) and were approved by the Hallym's Medical Center Institutional Animal Care and Use Committee.

Male gerbils at 6 months (B.W., 66–75 g) were placed under general anesthesia with a mixture of 2.5% isoflurane in 33% oxygen and 67% nitrous oxide. A midline ventral incision was made in the neck, and both common carotid arteries were isolated, freed of nerve fibers, and occluded using non-traumatic aneurysm clips. Complete interruption of blood flow was confirmed by observing the central artery in retina using an ophthalmoscope. After 5 min of occlusion, the aneurysm clips were removed from the common carotid arteries. Restoration of blood flow (reperfusion) was directly observed under the ophthalmoscope. We maintained the body (rectal) temperature under free-regulating or normothermic (37 ± 0.5 °C) conditions with a rectal temperature probe (TR-100; YSI, USA) and thermometric blanket before, during the surgery, and after the surgery until the animals fully recovered from anesthesia. Sham-operated animals served as controls: these sham-operated animals were subjected to the same surgical procedures except that the common carotid arteries were not occluded.

To confirm the delayed neuronal death in the hippocampus after transient forebrain ischemia, at designated times (2 days, 3 days, 4 days, and 7 days after the surgery), sham-operated (n = 3 at each time point) and operated animals (n = 5 at each time point) were sacrificed for neuronal nuclei (NeuN) immunohistochemistry. For immunohistochemistry for Rip, at designated times (30 min, 3 h, 6 h, 12 h, 24 h, 2 days, 3 days, 4 days, 5 days, and 7 days after the surgery), sham-operated (n = 3 at each time point) and operated animals (n = 7 at each time point) were sacrificed. For histology, 30-μm thick coronal sections on a cryostat and the sections were prepared according to our previous method (Hwang et al., 2005).

To obtain the exact data in this study, all the tissues were simultaneously processed at the same moment in the same day. The sections were sequentially treated with 0.3% hydrogen peroxide (H_2O_2) in PBS for 30 min, incubated in 10% normal horse serum in PBS for 30 min, and in mouse anti-NeuN (Chemicon, diluted 1:1000) and diluted mouse anti-Rip (1:50, Hybridoma bank, USA) for 48 h at 4 °C, respectively.

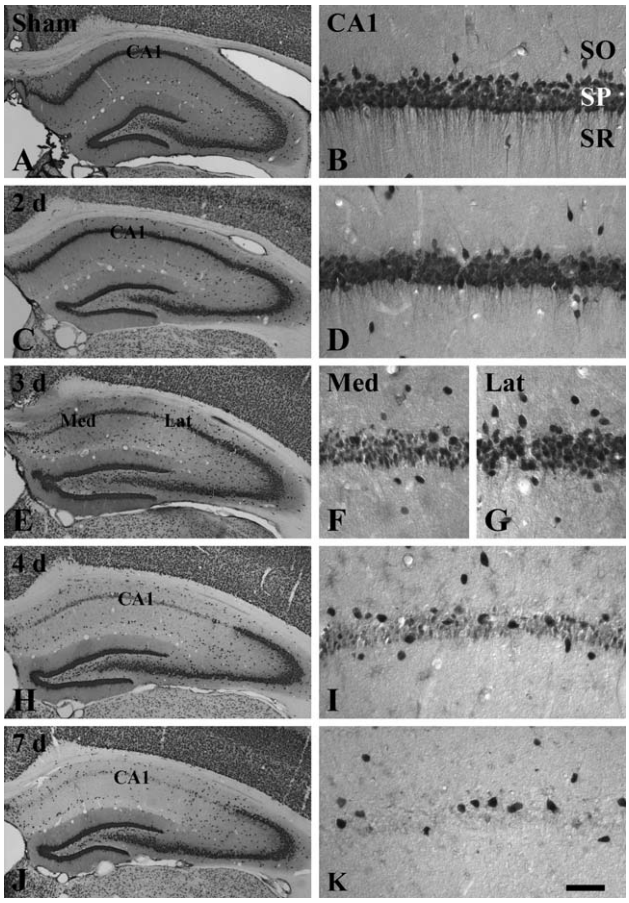

Fig. 1 – Immunohistochemistry for NeuN in the gerbil hippocampus of sham-operated (A and B) and operated groups (C-H) after transient forebrain ischemia. In the sham-operated group (A and B), NeuN-immunoreactive neurons are detected in all regions (A and B). Two days after ischemic insult (C and D), NeuN immunoreactivity in the CA1 region (CA1) is similar to that in the sham-operated group. Three days after ischemic insult (E–F), NeuN-immunoreactive pyramidal cells in the medial part (med) of CA1 show fast degeneration compared to those in the lateral part (lat). Four days after ischemia (H and I), a few NeuN-immunoreactive pyramidal cells are shown in CA1 due to delayed neuronal death of the CA1 pyramidal cells. Seven days after ischemia, CA1 pyramidal cells nearly disappear (J and K). SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum. Scale bars = 800 μm (A, C, E, H and J), 50 μm (B, D, F, G, I and K).