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The pattern of E2F1 and c-myb immunoreactivities in the CA1 region is different from those in the CA2/3 region of the gerbil hippocampus induced by transient ischemia

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Abstract

In this study, we examined transient ischemia-induced changes in transcription factor E2F1 and c-myb expressions in the gerbil hippocampus after 5 min of transient forebrain ischemia. E2F1 immunoreactivity significantly increased in the CA1 region 6–12 h after ischemia/reperfusion. c-myb immunoreactivity increased mainly in CA1 pyramidal cells with time by 12 h after ischemia. Thereafter, E2F1 and c-myb immunoreactivities significantly decreased compared to those in the 12 h post-ischemic group. Four days after ischemia/reperfusion, E2F1 and c-myb immunoreactivities were detected in non-pyramidal cells. Ten days after ischemia, c-myb immunoreactivity increased again: at this time, astrocytes as well as non-pyramidal cells showed E2F1 and c-myb immunoreactivities. In the CA2/3 region, E2F1 and c-myb immunoreactivities mainly changed in non-pyramidal cells, and 10 days after ischemia, c-myb immunoreactivity was not expressed in astrocytes. In conclusion, E2F1 and c-myb significantly alter in pyramidal cells and express in astrocytes in the gerbil hippocampal CA1 region after transient ischemia. These results indicate that E2F1 and c-myb in the CA1 region after ischemic damage may be associated with delayed neuronal death.
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1. Introduction

Vertebrate myb transcription factors comprise a small family of regulatory proteins with critical roles in cell proliferation and differentiation [1]. Among these myb families, c-myb is a transcription factor employed in hematopoietic system and gastrointestinal tract to regulate exquisite balance between cell division, differentiation and survival [2,3]. In addition, c-myb is also expressed at high level in immature thymocytes and may be a regulator of T cell differentiation, as suggested by the partial block of thymopoiesis in transgenic mice expressing a c-myb dominant-negative construct [4].

A relatively low basal level of c-myb protein is detected in control neuronal cultures [5] and in the mouse hippocampus [6]. In addition, it is reported that the low level of c-myb mRNA reveals in many different neuron types in the adult rat brain [7]. Neuronal apoptosis is developed by the rapid induction of c-myb protein in cortical neurons, sympathetic neurons and neuronal PC12 cells in response to DNA damage or NGF withdrawal and by the forced expression of this protein [5].

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A few studies on expression and change in transcription factor c-myb have been reported in the brain. Shin et al. [8] first investigated changes in c-myb immunoreactivity in the central nervous system of the transgenic mice expressing a human copper/zinc superoxide dismutase (Cu/Zn SOD) mutation. In addition, it has been reported that the induction of c-myb is delayed and protracted in the mouse brain following kainic acid induced seizure [6]. They suggested that a role of c-myb signal pathway in reactive gliosis in mice with kainic acid may induce seizure.

E2 promoter binding factor (E2F) transcription factors are cell-cycle regulatory molecules with key roles in neuronal survival and death [9,10]. E2F-regulated genes derepressed in neurons by apoptotic stimuli are the transcription factors b- and c-myb [11]. The over-expression of mybs induces neuronal death [11], whereas the down-regulation of mybs protects neurons from death [10].

However, ischemia-induced changes in E2F and c-myb have not been investigated in ischemic brain. Transient forebrain ischemia arises in humans as a consequence of cardiac arrest or cardiac surgery. This is induced experimentally in animals, and leads to selective and delayed neuronal death, particularly in pyramidal cells of the hippocampal CA1 region [12–15]. Therefore, we examined chronological changes in E2F1 and c-myb in the gerbil hippocampus after 5 min of transient forebrain ischemia to elucidate the possible correlation between chronological neuronal derepression via E2F1, c-myb expression and delayed neuronal death/neuronal survival in the gerbil hippocampus.

2. Materials and methods

2.1. Experimental animals

The present 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 conventional state under adequate temperature (23 °C) and humidity (60%) control with a 12-h light/12-h dark cycle, and provided with free access to water and food. Procedures involving animals and their care conformed with the 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. All experiments were conducted to minimize the number of animals used and suffering caused.

2.2. Induction of transient forebrain ischemia

Male Mongolian gerbils were used at 6 months (B.W., 70–75 g) of age. The animals were anesthetized with a mixture of 2.5% isoflurane in 33% oxygen and 67% nitrous oxide. A midline ventral incision was then made in the neck, and both common carotid arteries were isolated, freed of nerve fibers, and occluded using non-traumatic aneurysm clips. The complete interruption of blood flow was confirmed by observing the central artery in retinae using an ophthalmoscope. After 5 min of occlusion, the aneurysm clips were removed from the common carotid arteries. The restoration of blood flow (reperfusion) was observed directly using 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 a thermometric blanket before, during and after the surgery until the animals completely 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 [16].

2.3. Tissue processing for histology

For histology, sham-operated ($n=5$ at each time point) and operated animals ($n=7$ at each time point) at designated times (15 min, 30 min, 3 h, 6 h, 12 h, 2 days, 3 days, 4 days, 5 days, 7 days and 10 days after the surgery) were sacrificed. The animals were anesthetized with pentobarbital sodium and perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brains were removed and postfixed in the same fixative for 6 h. The brain tissues were cryoprotected by infiltration with 30% sucrose overnight. Thereafter the frozen tissues were serially and transversely cut to 30 μm thickness on a cryostat and then collected into six-well plates containing PBS.

2.4. Cresyl violet staining

To confirm the delayed neuronal death of CA1 pyramidal cells, the sections were stained with cresyl violet acetate. In brief, the sections were mounted on gelatin-coated microscopy slides. Cresyl violet acetate (Sigma, MO) was dissolved at 1.0% (w/v) in distilled water, and glacial acetic acid was added to this solution. Before and after staining for 2 min at room temperature, the sections were washed twice in distilled water. The fixed brain tissues were dehydrated by immersing for 2 h in 50%, 70%, 80%, 90%, 95% and 100% ethanol baths in succession at room temperature. After dehydration, the sections were mounted with Canada Balsam (Kato, Japan).

2.5. Immunohistochemistry for E2F1 and c-myb

To ensure that immunohistochemical data were comparable between groups, the free-floating sections were carefully processed under the same conditions. The sections were sequentially treated with 0.3% hydrogen peroxide (H_2O_2) in PBS for 30 min and 10% normal horse serum in 0.05 M PBS for 30 min. They were next incubated with

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