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Difference of fibroblast growth factor receptor 1 expression among CA1-3 regions of the gerbil hippocampus after transient cerebral ischemia

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ABSTRACT

Fibroblast growth factors are important regulators of neuronal development. In this study, we observed fibroblast growth factor receptor 1 (FGFR1) immunoreactivity and its protein levels in the hippocampus proper (CA1-3 regions) of the gerbil at various time points after ischemia/reperfusion. In the sham-operated group, FGFR1 immunoreaction was not detected in the hippocampus proper. FGFR1 immunoreaction was first detected in non-pyramidal neurons in the CA1-3 region at 12 h and 1 day after ischemia/reperfusion. From 2 days after ischemia/reperfusion, FGFR1 immunoreaction was found in astrocytes, not in microglial cells, in the CA1 region: FGFR1 immunoreactivity and the number of astrocytes were significantly increased at 5 days post-ischemia. Western blot analysis revealed that FGFR1 protein levels were also increased from 1 day after ischemia/reperfusion. These results indicate that increase of FGFR1 in astrocytes of the ischemic CA1 region may be associated with gliosis followed by delayed neuronal death.

1. Introduction

Transient cerebral ischemia produced by transient deprivation of blood flow to the brain, as well as hippocampal neurons established the model of oxygen–glucose deprivation, leads to increased Ca^{2+} influx due to membrane depolarization [1,2]. In the brain, the hippocampus is one of vulnerable regions to transient cerebral ischemia. It is widely accepted that transient cerebral ischemia leads to delayed neuronal death of pyramidal neurons in the hippocampal CA1 region in adult gerbils [3,4]. Death of the CA1 pyramidal neurons following cerebral ischemia causes a variety of neurological dysfunction such as depression and memory deficits [5–9].

Fibroblast growth factors (FGFs) are important regulators of neuronal development with potent broad-spectrum neurotrophic and mitogenic activities in mesoderm- and neuroectoderm-derived cell lines [10–12]. Disruption of the normal expression of FGF2 and FGF receptor 1 (FGFR1) is thus likely to have profound effects on CNS development, maintenance and repair [13,14]. It has been reported that FGF2 was up-regulated in various pathological disease such as kainite-induced seizure [15], depression [16] and fimbria-fornix transection [17] as well as ischemic damage [18–20].

It was reported that FGFR-1, FGFR-2 and FGFR-3 were expressed in the dentate gyrus of the hippocampus both during embryogenesis and throughout adulthood [21]. Several researchers have reported that brain ischemic insults increase FGF2 expression in the hippocampus [18–20]. However, there are few reports about the expression of FGFR1 in the hippocampus proper (CA1-3 regions) after transient cerebral ischemia. In the present study, therefore, we investigated the chronological changes of FGFR1 immunoreactivity and its protein levels in the hippocampal CA1-3 regions after 5 min of transient cerebral ischemia in gerbils.

2. Materials and methods

2.1. Experimental animals

The progeny of male Mongolian gerbils (*Meriones unguiculatus*) were obtained from the Experimental Animal Center, Hallym

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University, Chuncheon, South Korea. Gerbils were used at 6 months (B.W., 65–75 g) of age. 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. The procedures for handling animals and their care conformed to the guidelines that 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). All of the experiments were conducted to minimize the number of animals used and suffering caused by such procedures.

2.2. Induction of transient cerebral ischemia

The animals were anesthetized with a mixture of 2.5% isoflurane (Baxtor, Deerfield, IL) in 33% oxygen and 67% nitrous oxide. Bilateral common carotid arteries were isolated 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 body (rectal) temperature under free-regulating or normothermic (37 ± 0.5 °C) conditions was monitored with a rectal temperature probe (TR-100; Fine Science Tools, Foster City, CA) and maintained using a thermometric blanket before, during and after the surgery until the animals completely recovered from anesthesia. Thereafter, animals were kept on the thermal incubator (Mirae Medical Industry, Seoul, South Korea) to maintain the body temperature of animals until the animals were euthanized. Sham-operated animals were subjected to the same surgical procedures except that the common carotid arteries were not occluded.

2.3. Tissue processing for histology

For the histological analysis, sham-operated and ischemia-operated animals were anesthetized with chloral hydrate (30 mg/kg, i.p.) 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, frozen tissues were

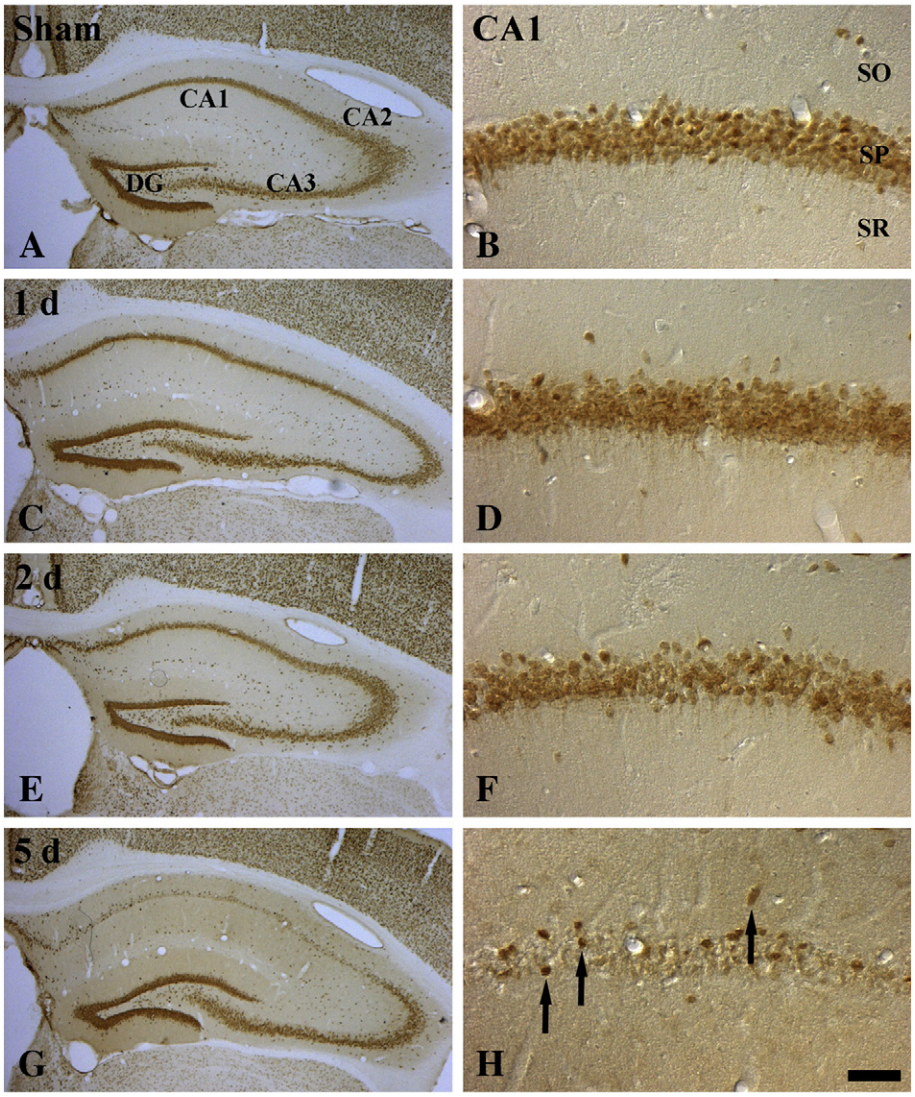

Fig. 1. NeuN immunohistochemistry of the hippocampus in sham- (A and B) and ischemia-operated groups 1 day (C and D), 2 days (E and F) and 4 days (G and H) after ischemia/reperfusion. Two days after ischemia/reperfusion, $NeuN^+$ pyramidal neurons in the CA1 region are similar to those in the sham-operated group. Four days after ischemia/reperfusion, $NeuN^+$ pyramidal neurons in the CA1 region show "delayed neuronal death." "Arrows" indicate non-pyramidal neurons. SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum. Scale bars = 250 μm (A, C, E and G), 50 μm (B, D, F and H).