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Research report

Tyrosine kinase A but not phosphacan/protein tyrosine phosphatase-ζ/β immunoreactivity and protein level changes in neurons and astrocytes in the gerbil hippocampus proper after transient forebrain ischemia

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Abstract

In the present study, ischemia-related changes in tyrosine kinase A (trkA) and phosphacan/protein tyrosine phosphatase-ζ/β (PTP-ζ/β) immunoreactivities and protein contents were examined in the hippocampus proper after transient forebrain ischemia for 5 min in a gerbil model. Our investigations showed that ischemia-induced changes occurred in trkA immunoreactivity in the hippocampal CA1 region, but not in the CA2/3 region of the hippocampus proper. In the sham-operated group, trkA immunoreactivity was barely detectable. trkA immunoreactivity increased from 30 min after ischemia and peaked at 12 h. Four days after ischemic insult, trkA immunoreactivity was observed in GFAP-immunoreactive astrocytes in the strata oriens and radiatum. In addition, we found that ischemia-related changes in trkA protein content were similar to immunohistochemical changes. On the other hand, PTP-ζ/β immunoreactivities in the hippocampus proper were unaltered by forebrain ischemia. These results suggest that chronological changes of trkA after transient forebrain ischemia may be associated with an ischemic damage compensatory mechanism in CA1 pyramidal cells.

Theme: Disorders of the nervous system
Topic: Ischemia

Keywords: Transient forebrain ischemia; Hippocampal CA1 region; Pyramidal cells; Astrocytes; Tyrosine kinase A; Phosphacan/protein tyrosine phosphatase-ζ/β; Gerbil

1. Introduction

The neurotrophins are important protective factors against ischemic damage in surviving neurons. Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3, and neurotrophin-4/5 regulate the survival, development, and maintenance of specific functions in different populations of neurons [35]. These neurotrophic factors exert their biological activities by activating two unrelated types of transmembrane receptors. The first type is a low-affinity NGF receptor, known as a low-affinity neurotrophin receptor, which binds neurotrophins with varying affinity and does not possess intrinsic tyrosine kinase activity [1,29]. Members of the second type are high-affinity NGF receptors that specifically bind individual neurotrophins. These receptors are members of the protein tyrosine kinase (trk) family of tyrosine kinases, and include trkA, trkB, and trkC [10,22].

The effects of growth factors such as NGF are mediated by high-affinity receptor protein tyrosine kinase (trkA)

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expressed on target cells. Tyrosine phosphorylation is a regulatory mechanism that is involved in various types of adaptive responses, such as cell growth and differentiation [20], synaptic plasticity [2], cell repair [18], and cell toxicity [25] in the brain. And, dephosphorylation by protein tyrosine phosphatases (PTPs) is thought to play an important role in regulating this signaling pathway [5]. Moreover, PTPs and trkA increase in the cerebral cortex following ischemic insult [6,16,31,34].

Transient forebrain ischemia produced by depriving the brain of circulating blood, results in the insidious delayed degeneration of specific vulnerable neurons within the hippocampal CA1 region [3,12,27,28]. Brain ischemia and reperfusion damage destroys trk/PTP homeostasis [26], which increases the tyrosine phosphorylation of *N*-methyl-D-aspartate receptor 2A (NR2A), leading to Ca^{2+} influx [36], intracellular calcium dependent kinase cascade activation, and eventually Src activation [17]. Although the expressions of trkA and PTPs may contribute to the susceptibility of neurons to ischemic insult [6,16,31,34], no detailed study has been conducted on ischemia-related trkA and PTPs changes in the hippocampus after ischemic insult.

Therefore, in the present study, we examined the spatial and temporal changes of the expressions of trkA and PTP-ζ/β in the gerbil hippocampus after 5 min of forebrain ischemia.

2. Materials and methods

2.1. Experimental animals

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. All experiment was conducted to minimize the number of animals used and suffering.

2.2. Induction of transient forebrain ischemia

Male Mongolian gerbils (*M. unguiculatus*) weighing 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. 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 arteries in eyeballs using an ophthalmoscope. After 5 min of occlusion, the aneurysm clips were removed from both of the common carotid arteries. Restoration of blood flow (reperfusion) was observed directly under the ophthalmoscope. Sham-operated animals were subjected to the same surgical procedures except that common carotid arteries were not occluded. Body temperature was monitored and maintained at 37 ± 0.5 °C during the surgery and during the immediate postoperative period until the animals recovered fully from anesthesia. At the designated reperfusion times, the sham-operated and operated animals were killed for immunohistochemical and Western blot study [9,11,14]. 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 PB, pH 7.4) at 30 min, 3 h, 6 h, 12 h, 24 h, 2 days, 3 days, and 4 days ($n = 10$ at each time point) after induction of ischemia. Sham-operated animals served as controls ($n = 10$).

2.3. Immunohistochemistry for trkA and PTP-ζ/β

Five animals in each group were used in immunohistochemical study. Brains were removed, and post-fixed in the same fixative for 6 h. The brain tissues were cryoprotected by infiltration with 30% sucrose overnight. Thereafter, the tissues were frozen and sectioned with a cryostat at 30 μm and consecutive sections were collected in six-well plates containing PBS.

The sections were sequentially treated with 0.3% hydrogen peroxide (H_2O_2) in PBS for 30 min and 10% normal goat serum in 0.05 M PBS for 30 min. The sections were next incubated with rabbit anti-trkA antibody (diluted 1:200, Santa Cruz Biotech, USA) or mouse anti-PTP-ζ/β antibody (2B49 or 3F8, diluted 1:100, Developmental Studies Hybridoma Bank, USA) overnight at room temperature. Thereafter, the tissues were exposed to goat anti-rabbit IgG or goat anti-mouse IgG and streptavidin peroxidase complex (Vector, USA). The sections were visualized with 3,3′-diaminobenzidine in 0.1 M Tris buffer and mounted on the gelatin-coated slides.

To establish the specificity of primary antibody, procedure included the omission of the primary antibody, goat anti-rabbit IgG, the substitution of normal horse serum or normal rabbit serum for the primary antibody. As a result, immunoreactivity disappeared completely in tissues. All experiment procedures in the present study were performed under the same circumstance and in parallel.

2.4. Double immunofluorescence staining for trkA/GFAP or trkA/OX-42

To confirm the glial type containing trkA immunoreactivity, double immunofluorescence staining for rabbit anti-trkA (1:250)/mouse anti-glial fibrillary acidic protein (GFAP) (1:250, Serotec) or rabbit anti-trkA/mouse anti-