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BRAIN RESEARCH

Research Report

Inhibition of cerebral ischemia/reperfusion-induced injury by adenovirus expressed C-terminal amino acids of GluR6

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ABSTRACT

GluR6 kainate receptor subunit is largely expressed in hippocampus of brain regions and plays an important role in brain ischemia/reperfusion-mediated neuronal cell death. Our previous researches have shown that cerebral ischemia/reperfusion could facilitate the assembly of GluR6 and postsynaptic density protein 95(PSD95) as well as mixed lineage kinase 3(MLK3) and further induce the activation of c-Jun NH2-terminal kinase 3(JNK3), leading to neuronal death of hippocampal CA1. Here, we show that over-expression of C-terminal amino acids of GluR6 can interrupt the combination of GluR6 with PSD95, inhibit the assembly of GluR6·PSD-95·MLK3 signaling module, suppress the activation of JNK3 and the downstream signaling pathway. Thus, our results imply that over-expression of C-terminal amino acids of GluR6 induce neuroprotection against ischaemic brain injury in rat hippocampal CA1 region via suppressing proapoptosis signaling pathways, which can be an experimental foundation for gene therapy of stroke.

1. Introduction

Excitotoxicity is regarded as the main mechanism leading to neuronal death in stroke. The ionotropic glutamate receptors, which are involved in the neuronal excitotoxicity, are pharmacologically divided into *N*-methyl-D-aspartate (NMDA),α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) and kainate (KA) receptors. Since NMDA and AMPA receptors have been widely researched, the physiological roles of KA receptors have begun to be delineated. KA receptors have five different subunits identified: GluR5, GluR6, GluR7, KA1, and KA2, which are expressed in distinct patterns in different areas of the hippocampus (Bureau et al., 1999). GluR6 is largely expressed in brain regions involved in learning and memory, for example the CA1 and CA3 of the hippocampus (Darstein et al., 2003). Fisahn (2005) concluded that the KAR GluR6 was involved in mediating kainate-induced excitation in the hippocampal network. GluR6 kainate receptor subunit is a major subunit which can take part in native heteromeric complexes by co-assembly with other kainate receptor

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Abbreviations: Ad, adenovirus; GluR6, glutamate receptor 6; KA, kainic acid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MKK4/7, mitogen-activated kinase kinase 4/7; MLK3, mixed lineage kinase 3; NMDA, N-methyl-D-aspartate; PSD-95, postsynaptic density protein 95; SD, Sprague-Dawley

[1] The first two authors contribute equally to this work.

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subunits (Paternain et al., 2000; Martin et al., 2007). Each GluR6 subunit is composed of a long extra-cellular N-terminal glutamate-binding pocket domain, which includes S1 region in cooperation with the S2 region, three trans-membrane domains (TM1, TM3 and TM4), a re-entrant membrane loop (M2), and of C-termini on the cytoplasmic side (Kornreich et al., 2007). Studies have shown that GluR6 C terminus could be disturbed to influence assembling of the downstream proteins. When the C-terminal region of GluR6 is deleted, the protein is not associated with the synapse-associated protein PSD95/SAP90 (Strutz-Seebohm et al., 2006). The last four residues of the GluR6 C terminus (E-T-M-A-oh) were

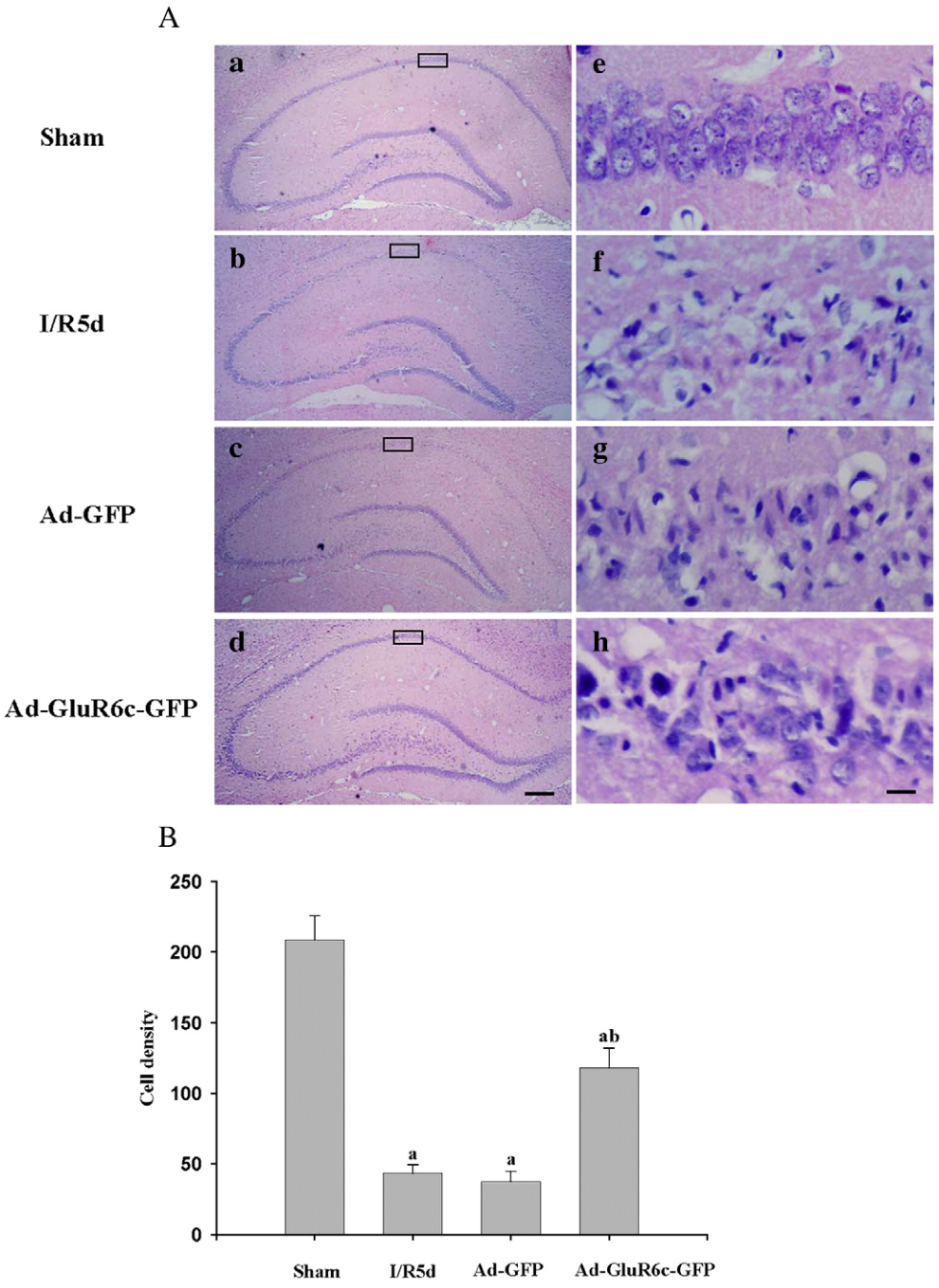

Fig. 1 – Over-expression of C-terminal amino acids of GluR6 improves the survival SD rat hippocampal CA1 neurons induced by 15 min of ischemia followed by 5 days of reperfusion. (A) Example of cresyl violet-stained sections of the hippocampi of sham operated rats (a, e), rats subjected to 15 min of ischemia followed by 5 days of reperfusion (b, f), rats subjected to 15 min of ischemia followed by 5 days of reperfusion with administration of the Ad-GFP (c, g) and Ad-GluR6c-GFP 5 days before ischemia (d, h). Data were obtained from six independent animals and the results of a typical experiment are presented. Boxed areas in left column are shown at higher magnification than in right column. a, b, c, d: ×40; e, f, g, h: ×400. Scale bar in d = 200 mm; scale bar in h = 10 mm. (B) Cell density was expressed as the number of cells per 1 mm length of the CAI pyramidal cells counted under a light microscope. Data were the mean ± S.D.(n = 6). (a) $P < 0.05$ versus sham; (b) $P < 0.05$ versus Ad-GFP groups.