Available online at www.sciencedirect.com

SCIENCE DIRECT

Brain Research 1061 (2005) 57 – 66

BRAIN RESEARCH

www.elsevier.com/locate/brainres

Research Report

Activation of c-Jun NH_2-terminal kinase 3 is mediated by the GluR6·PSD-95·MLK3 signaling module following cerebral ischemia in rat hippocampus

Hui Tian [b,1], Quan-Guang Zhang [a,1], Guang-Xuan Zhu [a], Dong-Sheng Pei [a], Qiu-Hua Guan [a], Guang-Yi Zhang [a,*]

[a] *Research Center for Biochemistry and Molecular Biology, Xuzhou Medical College, 84 West Huai-hai Road, Xuzhou, Jiangsu 221002, PR China*
[b] *School of Life Science, Xuzhou Normal University, 29 Shanghai Road of Tongshan district, Xuzhou, Jiangsu 2211162, PR China*

Accepted 1 September 2005

Abstract

Kainate receptor glutamate receptor 6 (GluR6) binds to the postsynaptic density protein 95 (PSD-95), which in turn anchors mixed lineage kinase 3 (MLK3) via SH3 domain in rat brain tissue. MLK3 subsequently activates c-Jun NH_2-terminal kinase (JNK) via MAP kinase kinases (MKKs). We investigated the association of PSD-95 with GluR6 and MLK3, MLK3 autophosphorylation, the interaction of MLK3 with JNK3, and JNK3 phosphorylation following cerebral ischemia in rat hippocampus. Our results indicate that the GluR6·PSD-95·MLK3 complex peaked at 6 h of reperfusion. Furthermore, MLK3 autophosphorylation and the interaction of MLK3 with JNK3 occurred with the alteration of GluR6·PSD-95·MLK3 signaling module. To further prove whether JNK3 activation in ischemic hippocampus is mediated by GluR6·PSD-95·MLK3 signaling pathway, the AMPA/KA receptor antagonist 6,7-dinitroquinoxaline-2, (1*H*, 4*H*)-dione (DNQX), the GluR6 antagonist 6,7,8,9-Tetrahydro-5-nitro-1*H*-benz[g]indole-2,3-dione-3-oxime (NS102), the AMPA receptor antagonist 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzo diazepine (GYKI52466), and the NMDA receptor antagonist ketamine were given to the rats 20 min prior to ischemia. Our findings indicate that both DNQX and NS102 significantly attenuated the association of PSD-95 with GluR6 and MLK3, MLK3 autophosphorylation, interaction of MLK3 with JNK3, and JNK3 phosphorylation, while GYKI52466 and ketamine had no effect. Moreover, administration of NS102 before cerebral ischemia significantly increased the number of the surviving hippocampal CA1 pyramidal cells at 5 days of reperfusion. Consequently, GluR6, one subunit of kainate receptor, plays a critical role in inducing JNK3 activation after ischemic injury.

Theme: Disorders of the nervous system
Topic: Ischemia

Keywords: c-Jun NH2-terminal kinase 3; Kainate receptor; Glutamate receptor 6; Postsynaptic density protein 95; Mixed lineage kinase 3; Cerebral ischemia

1. Introduction

Glutamate receptors (GluRs) are classified into two subgroups: ionotropic receptors and metabotropic receptors. Ionotropic glutamate receptors can be divided into two classes, *N*-methyl-D-aspartate (NMDA) receptors (NR1, NR2A–NR2D, NR3A–B) and non-NMDA receptors. Non-NMDA receptors can be further subdivided into α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (GluR1–4) and kainate acid (KA) receptors (GluR5–GluR7, KA1 and KA2) [1,12,32]. Ionotropic glutamate receptors mediate excitatory synapse transmission in the mammalian central nervous system and play a central role in learning and memory. Furthermore, calcium

* Corresponding author. Fax: +86 516 574 8486.
E-mail address: gyzhang@xzmc.edu.cn (G.-Y. Zhang).
[1] The first two authors contributed equally to this work.

0006-8993/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.09.001

influx through ionotropic glutamate receptors modulates many physiological functions in the central nervous system. Also note that considerable evidence suggests that glutamate receptors are also involved in neuronal death after ischemic insults [25,33]. Excessive activation of glutamate receptors can cause excitotoxicity and cell death by increasing transcellular calcium concentration. Thus, neuroprotective action has mainly focused on blocking excitotoxicity induced by the excitatory transmitter glutamate and Ca^{2+} overload in neurons. The NMDA receptors are highly Ca^{2+}-permeable, and cerebral ischemia induces enhancement of the expression of Ca^{2+}-permeable AMPA receptors [25]. However, this view that excitotoxicity with attendant neuronal Ca^{2+} overload is the predominant mechanism underlying ischemic brain injury faces challenge due to negative results from several recent trials with antagonists of NMDA receptor [16]. Previous studies report GluR6 knockout mice are more resistant to kainate-induced seizures and excitotoxic neuronal death in the hippocampus [22]. Consequently, we are particularly interested in establishing whether and how ischemic brain damage is mediated by KA receptor GluR6 subunit.

The postsynaptic density protein 95 (PSD-95) is a scaffold protein characterized by the presence of several protein binding domains including three N-terminal PDZ, a signal Src homology region 3 domain, and a C-terminal guanylate kinase-like domain [3,15,23]. The PDZ domains are known to bind to the C-terminus of NMDA receptor NR2 and KA receptor GluR6 subunit, and these interactions are very crucial for the clustering of NMDA receptors and KA receptors in the postsynaptic membrane [8,21,40]. In addition, PSD-95 also interacts with some cytoplasmic signaling proteins. For example, PSD-95 can connect NR2 with neuronal nitric oxide synthase (nNOS) [30]. Some studies indicate that PSD-95, GluR6, and MLK3 form an association, and GluR6-deficient mice show strong resistance to kainate-induced seizures [22,31]. Therefore, PSD-95 plays a critical role for GluR6-mediated MLK3 activation and neuronal excitotoxicity induced by kainate in cell lines.

MLK3, a member of mixed lineage kinase family, is composed of an N-terminal SH3 domain, a middle kinase domain, and a C-terminal proline-rich region [7]. It is proven that C-terminal proline-rich region in MLK3 binds to SH3 domain of PSD-95 [31]. MLKs are the direct activators of MKK4/7 which subsequently phosphorylate and activate JNKs. The c-Jun NH2-terminal kinase (JNKs), or stress-activated protein kinase (SAPK), belongs to the mitogen-activated protein kinase (MAPK) family of proline-directed serine/threonine kinase that participates in intracellular signaling pathway by transmission of signals from the plasma membrane to the nucleus. Additionally, JNKs are critically involved in the apoptosis of NGF-deprived PC12 cells [2,6]. It is important to note that current studies demonstrate that the knockout mice of JNK3 gene resist the kainate-induced seizure [41], just as GluR6-deficient mice. Although JNK1/2 are widely expressed in a variety of tissues, JNK3 is found predominately in neurons [11]. On the other hand, studies from both our group and others show that JNKs are activated and implicated in neuronal degeneration in response to ischemic insult [9,14]. However, JNKs' specific upstream activator and signaling pathway in cerebral ischemia remain complicated.

Although studies from Savinainen et al. [31] show that PSD-95 plays a critical role in GluR6-mediated JNK activation and excitotoxicity by anchoring MLKs to the GluR6 complex, the results were mainly obtained from cell lines experiment and kainate stimulation. Meanwhile, our preliminary studies show that the MLK3 and JNK3 activation induced by cerebral ischemia–reperfusion in animal models is related to the activation of non-NMDA receptors, but not NMDA receptors [35,36]. Therefore, the present study was undertaken to investigate whether JNK3 activation in response to cerebral ischemia was mediated by GluR6 activation. We hypothesize that ischemic injury induced the formation and alteration of GluR6·PSD-95·MLK3 signaling module, subsequently activated MLK3 and JNK3, and finally mediated the neuronal death in hippocampus.

2. Experimental procedures

2.1. Animal surgical procedures

Adult male Sprague–Dawley (SD) rats weighing 250–300 g were purchased from the Shanghai Experimental Animal Center of the Chinese Academy of Science. The experiment procedures were approved by the local legislation for ethics of experiments on animals. Transient brain ischemia was induced by four-vessel occlusion [9,29]. Briefly, the rats were anesthetized by chloral hydrate (350 mg/kg, i.p.), their vertebral arteries were then electrocauterized, and their common carotid arteries were exposed. The rats were allowed to recover for 24 h. Ischemia was induced by occluding the common arteries with aneurysm clips. The rats which lost their righting reflex within 30 s, and their pupils were dilated and unresponsive to light, were selected for the experiments. The rats with seizures were discarded. An EEG was monitored to ensure isoelectricity after carotid artery occlusion. Carotid artery blood flow was restored by releasing the clips. Rectal temperature was maintained at about 37 °C during and 2 h after ischemia. Sham animals received the same surgical procedures except bilateral carotid arteries were not occluded.

2.2. Brain tissues and drug treatment

In order to study time courses of the association of PSD-95 with GluR6 and MLK3, MLK3 autophosphorylation, interaction of MLK3 with JNK3, and JNK3 phosphorylation, rats were decapitated immediately (0 min) and at different times of reperfusion (30 min, 3 h, 6 h, 1 day, 3