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Research Report

Calcium/calmodulin-dependent kinase II facilitated GluR6 subunit serine phosphorylation through GluR6-PSD95-CaMKII signaling module assembly in cerebral ischemia injury

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ABSTRACT

Although recent results suggest that GluR6 serine phosphorylation plays a prominent role in brain ischemia/reperfusion-mediated neuronal injury, little is known about the precise mechanisms regulating GluR6 receptor phosphorylation. Our present study shows that the assembly of the GluR6–PSD95–CaMKII signaling module induced by brain ischemia facilitates the serine phosphorylation of GluR6 and further induces the activation of c-Jun NH2-terminal kinase JNK. More important, a selective CaMKII inhibitor KN-93 suppressed the increase of the GluR6–PSD95–CaMKII signaling module assembly and GluR6 serine phosphorylation as well as JNK activation. Such effects were similar to be observed by NMDA receptor antagonist MK801 and L-type Ca^{2+} channel (L-VGCC) blocker Nifedipine. These results demonstrate that NMDA receptors and L-VGCCs depended-CaMKII functionally modulated the phosphorylation of GluR6 via the assembly of GluR6–PSD95–CaMKII signaling module in cerebral ischemia injury.

1. Introduction

Kainate (KA) receptors, one of the ionotropic glutamate receptors, mediate most excitatory synaptic transmission in the vertebrate central nervous system and play major roles in synaptic plasticity, and pathological processes such as epilepsy (Dingledine et al., 1999). Kainate receptors have five different subunits identified: GluR5, GluR6, GluR7, KA1, and KA2 based upon their high affinity for this compound, which express in distinct patterns in different areas of the hippocampus (Bureau et al., 1999). Kainate receptor GluR6 subunit is largely expressed in the CA1 and CA3 of the brain hippocampus involved in learning and memory (Darstein et al., 2003). GluR6-deficient mice exhibit resistance to neurotoxic effects induced by KA, indicating that the possibility that the KA receptor GluR6 subunit may mediate the excitotoxicity of glutamate (Mulle et al., 1998). The carboxylic terminal of GluR6 could bind to the PDZ1 domain of postsynaptic density protein 95 (PSD-95/SAP90)

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Abbreviations: CaMKII, Calcium/calmodulin-dependent kinase II; L-VGCC, L-type Ca^{2+} channel; JNK3, c-Jun N-terminal kinase 3; I/R, ischemia/reperfusion; ABC, avidin–biotin complex; PMSF, phenylmethylsulfonyl; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; DAB, 3,3′-diaminobenzidine and hydrogen peroxide

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through a specific interaction (Mehta et al., 2001). Recent studies indicate that cerebral ischemia/reperfusion could facilitate the assembly of GluR6 and 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 regions (Tian et al., 2005). Over-expression of C-terminal amino acids of GluR6 and PSD95 all can interrupt the combination of GluR6 with PSD95, inhibit the assembly and induce neuroprotection against ischemic brain injury. Thus, kainate receptor GluR6 exerts essential roles in brain ischemic injury.

Phosphorylation is thought to be the major manner of protein kinase activation, which contributing to regulate their function in cerebral ischemia. Kinase-dependent phosphorylation of ionotropic glutamate receptors occurs primarily on serine and threonine (Ser/Thr) residues via mutational studies, immunochemical assays, phosphoamino acid analysis and phosphopeptide mapping (McFeeters and Oswald, 2004). Ca^{2+}-induced enhancement of GluR6 receptor serine phosphorylation have been proved to be mediated by calcium/calmodulin-dependent kinase II (CaMKII) in cultured hippocampal neurons (Yakel et al., 1995), CaMKII activated via binding of Ca^{2+}/CaM is a family of Ser/Thr protein kinases known as CaM-kinases (CaMKs), which is present throughout the neuron and it phosphorylate Ser/Thr residues in their protein substrates to alter the functionality of those proteins, such as N-methyl-D-aspartic acid (NMDA) receptor subunit NR2B. Significantly, CaMKII constitutes the major protein of the postsynaptic density (PSD) in dendritic spines of excitatory neurons where it interacts with several proteins (Colbran, 2004). Interaction of CaMKII with the PSD is dynamic and regulated by binding of Ca^{2+}/CaM and the phosphorylation status of CaMKII-autophosphorylation of Thr286 promotes and stabilizes CaMKII binding to the PSD (Strack et al., 1997). Immunoprecipitation results suggest CaMKII interacts with PSD95 as well as GluR6 with PSD95 and subsequently activated JNK3 in ischemic hippocampal neurons (Tian et al., 2005; Yan et al., 2004). Based on the above results, we wonder that CaMKII facilitates the serine phosphorylation of GluR6 and finally mediated the neuronal death in hippocampus through the assembly of the CaMKII-GluR6-PSD95 signaling module.

Our results indicated that the GluR6 serine phosphorylation was in line with the interactions among CaMKII, GluR6 and PSD95 in ischemic hippocampal neurons. A selective CaMKII inhibitor KN-93, NMDA receptor antagonist MK801 and L-type Ca^{2+} channel (L-VGCC) blocker Nifedipine interrupted the assembly of GluR6–PSD95–CaMKII signaling module and decreased GluR6 serine phosphorylation as well as JNK3 activation. These data suggested that CaMKII is important for facilitating GluR6 serine phosphorylation.

2. Results

2.1. *The alteration of GluR6–PSD95–CaMKII signaling module and the GluR6 serine phosphorylation during reperfusion after cerebral ischemia*

To determine that whether CaMKII was concerned with GluR6 serine phosphorylation in rat brain ischemia, we first detected the assembly of GluR6–PSD95–CaMKII signaling module in different time points (sham, 0 min, 30 min, 3 h, 6 h, 12 h, and 1 day) of reperfusion after 15 min of ischemia. Endogenous CaMKII, GluR6, and PSD95 were immunoprecipitated from the hippocampal samples with three specific antibodies, respectively. As shown in Fig. 1, the antibodies of CaMKII and GluR6 could co-precipitate the other two proteins in the ischemia/reperfusion (I/R) groups. The sample proteins from the hippocampal CA1 regions were immunoprecipitated and immunoblotted with antibody against GluR6 serine phosphorylation, GluR6, respectively. As shown in Figs. 1A and B, the activation of GluR6 in I/R continuously elevated from 30 min of reperfusion, and peaked at 6 h of reperfusion, then fell at 1d. No significant corresponding band was detected. Importantly, we found that the trend of GluR6 serine phosphorylation level was in line with the alteration of GluR6–PSD95–CaMKII signaling module. These data confirmed the hypothesis we described above, which indicated that the definite existence and alteration of GluR6–PSD95–CaMKII signaling module and involved in GluR6 serine phosphorylation during reperfusion.

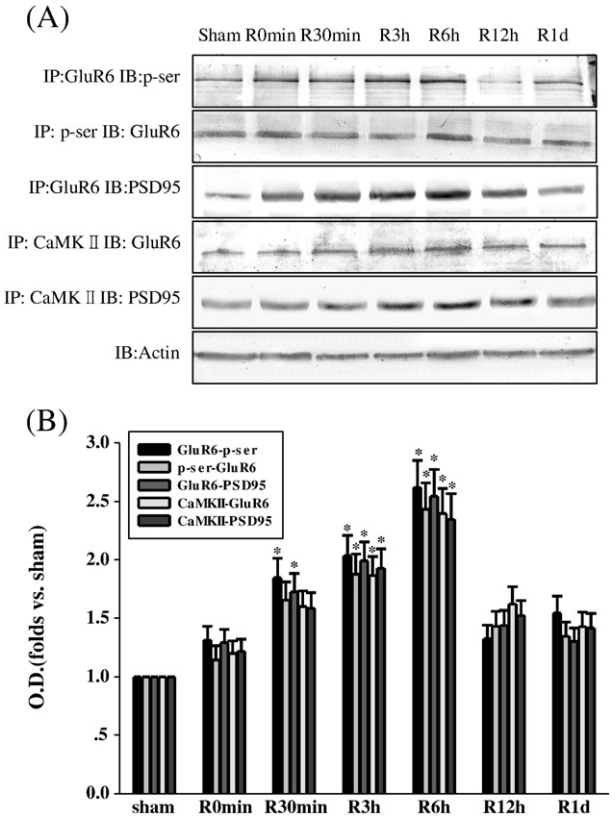

Fig. 1 – Changes level of GluR6 subunit serine phosphorylation and the GluR6–PSD95–CaMKII signaling module assembly in rat ischemia hippocampal CA1 regions. (A) Representative blots showing levels of the GluR6 subunit serine phosphorylation and the GluR6–PSD95–CaMKII signaling module assembly at different time of reperfusion after 15 min ischemia. (B) Semi-quantitative analysis of the GluR6 subunit serine phosphorylation and the GluR6–PSD95–CaMKII signaling module assembly at different times of reperfusion. Data were expressed as mean ± SD derived from three independent animals in each experiment group. *P < 0.05 vs. sham.