Influence of Hypoxia Preconditioning on NR2B and Its Phosphorylation at Tyrosine 1336 in the Mouse Hippocampus

Shaohan Zhang[1], Pu Zhang[2], Guo Shao[2, *]

[1]Traditional Chinese Medicine Department, Tianjin University of Traditional Chinese Medicine, Tianjin, China

[2]Center for Translational Medicine, Third People's Hospital of Longgang District, Shenzhen, China

Email address:

zhangshaohan_china@163.com (Shaohan Zhang), zhang_pu_china@163.com.com (Pu Zhang), shao_guo_china@163.com (Guo Shao)

[*]Corresponding author

Abstract

Previous studies have established that the N-methyl-D-aspartate (NMDA) receptor subunit 2B (NR2B) and its phosphorylation are key contributors to ischemic and hypoxic brain injury. Hypoxic preconditioning (HPC) serves as an endogenous mechanism to protect the brain from both ischemic and hypoxic damage. This study investigates the effects of HPC on NR2B and its phosphorylation at two specific tyrosine residues (1252 and 1336), mediated by Fyn kinase, in the hippocampus, both in vivo and in vitro. Animal and cellular models of HPC were created by exposing mice and the mouse hippocampal neuronal cell line HT22 to repeated hypoxia. The levels of NR2B and its phosphorylated forms (pY1336 NR2B and pY1252 NR2B) were assessed in HPC-treated hippocampi and HT22 cells using Western blotting and immunofluorescence. The distributions of NR2B, pY1336 NR2B, and pY1252 NR2B in synaptic (TxP) and extrasynaptic (TxS) components were analyzed via Western blotting. Additionally, markers of cellular apoptosis, caspase-3 and spectrin, were measured using Western blotting. HPC resulted in downregulation of NR2B and pY1336 NR2B levels in both the hippocampus and HT22 cells. The alterations in NR2B and pY1336 NR2B levels in extrasynaptic components mirrored those in the hippocampus and HT22 cells, while synaptic components exhibited an opposite trend, with increased levels following HPC. The downregulation of NR2B and pY1336 NR2B may contribute to the neuroprotective effects of HPC. Furthermore, their localization in synaptic and extrasynaptic sites may play distinct roles in neuroprotection.

Keywords

Hypoxic Preconditioning, NR2B, Synapse, Apoptosis