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Research Report

Pathway and gene ontology based analysis of gene expression in a rat model of cerebral ischemic tolerance

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ABSTRACT

Ischemic tolerance is a phenomenon whereby a sublethal ischemic insult [ischemic preconditioning (IPC)] provides robust protection against subsequent lethal ischemia. Activation of N-methyl-D-aspartate (NMDA) receptors and subsequent new gene transcription are required for tolerance. We utilized the NMDA antagonist, MK801, prior to the IPC stimulus to separate candidate genes from epiphenomenona. Rats were divided into four groups: vehicle/IPC (preconditioned), MK801/IPC (attenuated preconditioning), vehicle/sham (non-preconditioned), and MK801/sham (non-preconditioned). Hippocampi (5/group/time point) were harvested immediately after ischemia as well as 1, 4, and 24 h post-ischemia to profile gene expression patterns using microarray analyses. Extracted mRNAs were pooled and subsequently hybridized to Affymetrix arrays. In addition, groups of rats were sacrificed for Western blot analysis and histological studies. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and gene ontology (GO) analyses were used to identify functionally related groups of genes whose modulation was statistically significant, while hierarchical cluster analysis was used to visualize the fold expression within these groups. Significantly modulated pathways included: MAP kinase signaling pathway, Toll receptor pathway, TGF-β signaling pathways, and pathways associated with ribosome function and oxidative phosphorylation. Our data suggest that the tolerant brain responds to subsequent ischemic stress by partially downregulating inflammatory and upregulating protein synthesis and energy metabolism pathways.

1. Introduction

Ischemic tolerance (IT) is a phenomenon whereby a brief exposure to ischemic episodes results in ischemic preconditioning (IPC), providing neuroprotection against subsequent lethal ischemic insults (Dirnagl et al., 2003; Kirino, 2002; Schaller, 2005). Although the precise mechanisms by which tolerance is induced are not clear, the available evidence indicates that IPC

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involves new gene transcription and *de novo* protein synthesis (Truettner et al., 2002). Previous studies have shown that IPC modulates the transcription of genes associated with apoptosis, growth factors, free radical metabolism, and inflammation. These studies have revealed modulations in expression for genes involved in synthesis of proteins, cytokines, and ATP in

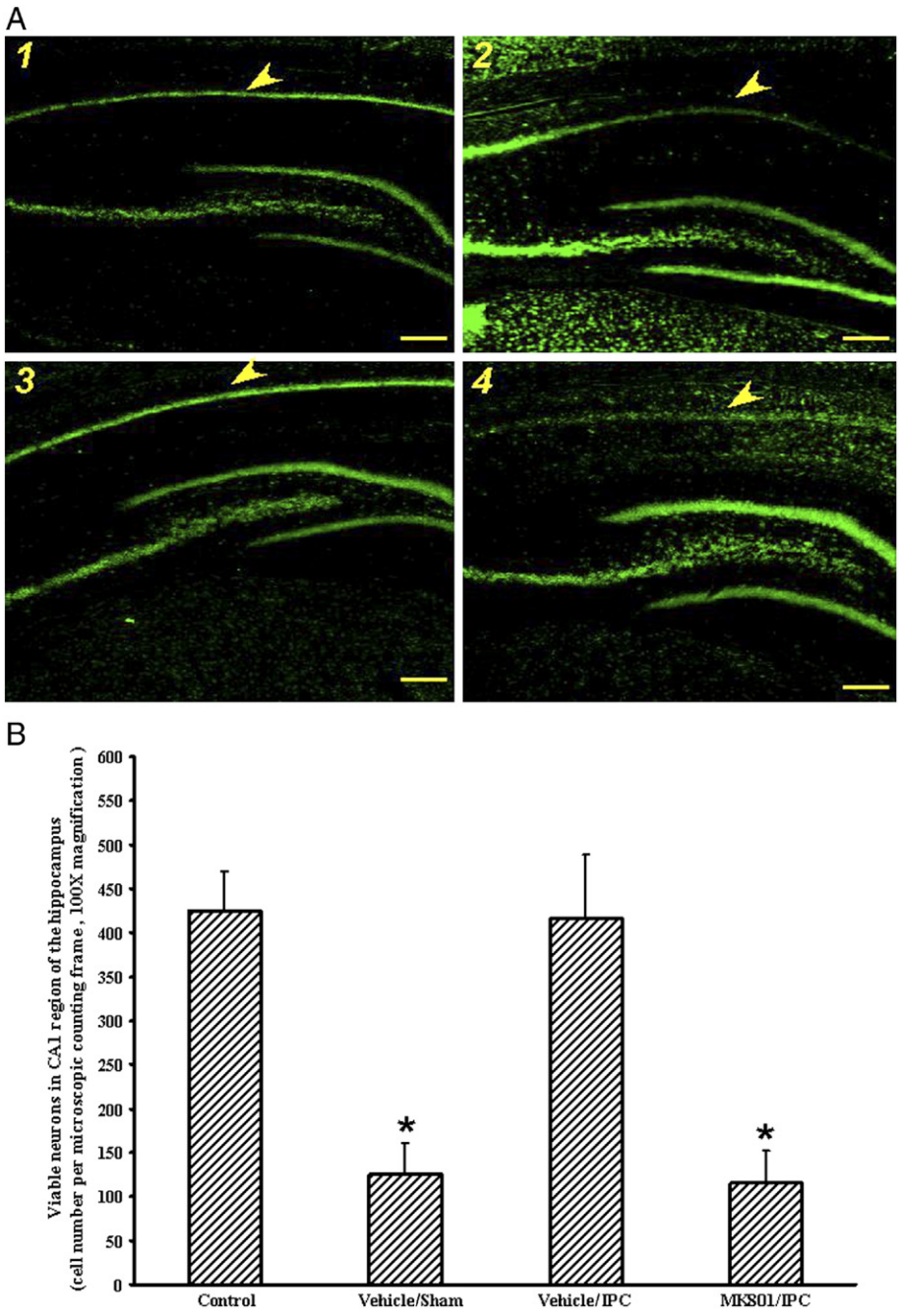

Fig. 1 – Validation of *in vivo* model. (A) Representative images demonstrating neuronal viability in the hippocampal CA1 sub-region. Coronal sections of the rat hippocampi were stained by NeuroTrace Nissl and observed under 50× magnification. 1. Nissl-stained section from a non-lesioned naive control showing broad and even distribution of CA1 pyramidal cells (arrows). 2. Lethal ischemia-induced marked loss of pyramidal neurons in CA1 region (arrows) in the vehicle/sham group. 3. IPC afforded nearly complete protection against subsequent lethal ischemia (arrows). 4. MK801 (3 mg/kg) pretreatment attenuated the protective effects of IPC (scale bar = 100 μm; magnification: 50×). (B) Quantitative analysis of neuronal loss in hippocampus CA1 region at baseline (control) and 7 days following lethal ischemia in vehicle/IPC, vehicle/sham, and MK801/IPC rats. The Nissl-stained sections were observed under an Olympus fluorescence microscope at 200× magnification. The number of surviving neurons in mediolateral segment of CA1 region was counted in three representative sections for each rat ($n = 3$). Neurons were counted only if their nuclei were completely within the margins of the counting frame. Data are expressed as mean ± SD ($n = 3$ per group). Statistical significance ($P < 0.05$) *versus* naive animals is indicated (*).