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Deletion of *Atf6α* enhances kainate-induced neuronal death in mice

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ABSTRACT

Excessive amount of L-glutamate in the brain causes neuronal damage in various pathological conditions including epilepsy and stroke. We previously reported that the 150-kDa oxygen-regulated protein (ORP150), a molecular chaperone in the endoplasmic reticulum (ER), inhibited the L-glutamate-induced neuronal death, at least partly, by improving Ca^{2+} homeostasis in the ER. In the present study, we analyzed the role of activating transcription factor 6α (ATF6α), an upstream transcriptional factor critical for the operation of the ER, using mouse intrahippocampal kainate (KA) injection model. Expression of *Hspa5*, which encodes the molecular chaperone 78 kDa glucose-regulated protein (GRP78), increased after KA injection in the wild type (WT) mice. Comparative analysis using WT and *Atf6α*$^{-/-}$ mice revealed that KA induced pronounced neuronal death in the CA3 region of *Atf6α*$^{-/-}$ mice. The enhanced neuronal death in *Atf6α*$^{-/-}$ mice was associated with reduced expression of molecular chaperones in the ER and significant induction of *c-fos* in the hippocampal neurons. Furthermore, an injection of dantrolene, an inhibitor of ryanodine receptor, partially rescued these effects in *Atf6α*$^{-/-}$ mice after KA injection. Our results suggest that ATF6α plays an important role in neuronal survival after KA-induced excitotoxicity through the regulation of Ca^{2+} response and neuronal activity.

1. Introduction

L-glutamate is the principal excitatory neurotransmitter in the central nervous system (CNS), but excessive amounts of L-glutamate can cause Ca^{2+}-dependent hyperactivation and subsequent neuronal damage, which is termed excitotoxicity. Accumulating evidence suggests the role of excitotoxicity in various neuropathological conditions such as epilepsy, stroke, trauma, and neurodegenerative diseases (Arundine and Tymianski, 2003; Lau and Tymianski, 2010). Mechanism of excitotoxicity has been studied extensively using kainate (KA), an agonist of L-glutamate receptors (Wang et al. 2005; Zheng et al. 2011). KA induces neuronal damage in the CNS, especially in the hippocampal CA1 and CA3 regions, where the KA receptors are abundantly expressed (Malva et al. 1998; Carta et al. 2014). KA-induced neuronal damage activates several cellular processes, including influx of Ca^{2+} (Sola et al. 2001), production of reactive oxygen species, mitochondrial dysfunction (Zheng et al., 2011), activation of astrocytes and microglial cells (Wang et al. 2004), and endoplasmic reticulum (ER) stress (Kitao et al. 2001).

ER stress is a condition where unfolded proteins are accumulated in the ER. This can be induced by various insults such as impairment of protein modifications, energy deprivation, and disturbance of Ca^{2+} homeostasis (Sokka et al. 2007; Yoshikawa et al. 2015). Under ER stress, cells try to maintain ER homeostasis through various processes collectively known as the unfolded protein response (UPR) (Ron and Walter, 2007). Mammalian cells

Abbreviations used: ATF6α, activating transcription factor 6α; CNS, central nervous system; CHOP, C/EBP homologous protein; DAPI, 4′,6-diamidino-2-phenylindole; ER, endoplasmic reticulum; FJC, Fluoro-Jade C; GFAP, glial fibrillary acidic protein; GRP78, 78 kDa glucose-regulated protein; GRP94, 94 kDa glucose-regulated protein; IRE1, inositol-requiring enzyme 1; KA, kainate; ORP150, 150-kDa oxygen-regulated protein; PERK, protein kinase R -like endoplasmic reticulum kinase; qRT-PCR, quantitative real-time reverse transcription PCR; RyR, ryanodine receptor; ssDNA, single-stranded DNA; UPR, unfolded protein response; XBP-1, X-box binding protein 1.

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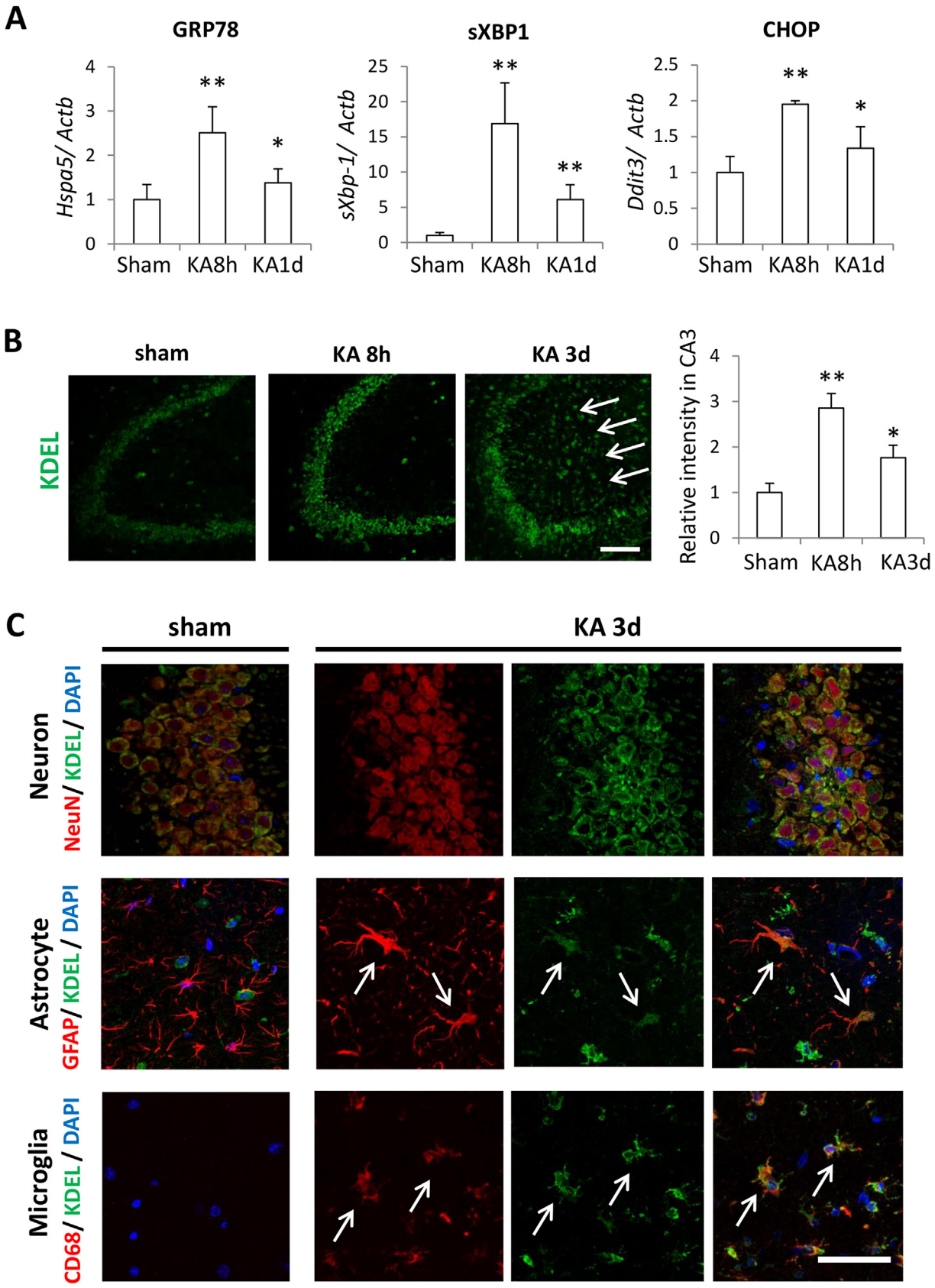

Fig. 1. Activation of the unfolded protein response in the hippocampus after kainite (KA) injection. A. Total RNA was purified from the hippocampus of KA-injected wild type (WT) mice and quantitative real-time reverse transcription PCR was performed with the indicated primers. *P < 0.05 and **P < 0.01 indicate statistical significance versus the sham injection. Values are presented as mean ± standard deviation (n = 3–4). B. Brain sections of WT mice were immunolabelled for KDEL. The right graph shows the intensity of KDEL-containing proteins such as the 78 kDa glucose-regulated protein (GRP78) in the pyramidal cells of the CA3 region. *P < 0.05 and **P < 0.01 indicate statistical significance versus the sham injection. Values are presented as mean ± standard deviation (n = 3). Scale bar: 100 μm. C. Brain sections of WT mice were immunostained for KDEL, NeuN, glial fibrillary acidic protein (GFAP), and CD68. The CA3 pyramidal cells (neuron) and the stratum radiatum (astrocyte and microglia) are shown. Scale bar: 50 μm.