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Research Report

NMDA preconditioning and neuroprotection in vivo: Delayed onset of kainic acid-induced neurodegeneration and c-Fos attenuation in CA3a neurons

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ABSTRACT

Intraventricular (icv) kainic acid (KA) causes an acute excitotoxic lesion to the CA3 region of rodent hippocampus. Recent evidence implicated *c-fos* gene in regulating neuron survival and death following an excitotoxic insult. In this study we attempted to prevent KA-induced damage in CA3 neurons with NMDA preconditioning, which produced a marked expression of *c-fos* in the hippocampus. NMDA (0.6–6 μg, icv) was injected to anesthetized rats alone or 1 h before KA (0.15 μg, icv). Following KA injection, vibratome sections were processed for immunohistochemistry/electron microscopy. c-Fos and Nissl staining were used to estimate the extent of neuronal excitation and damage, respectively. Quantitative evaluation of c-Fos-labeled cells showed significantly less c-Fos in CA3a than in neighboring CA3b and CA2 from 1 to 4 h after KA alone. Attenuation of expressed c-Fos in CA3a was accompanied by damage of neurons with more apoptotic than necrotic signs. NMDA preconditioning elevated CA3a c-Fos expression and at 1 and 2 h exceeded markedly that after KA alone. However, at 4 h after KA, NMDA-preconditioned c-Fos induction in CA3a diminished to the same level as that seen after KA alone. The onset of neuronal degeneration was delayed in similar way. While NMDA-induced c-Fos expression in CA3a could be blocked by MK-801 completely, MK-801 and CNQX were both without significant effect on KA-induced c-Fos expression and neuronal damage. In conclusion, inhibition of c-Fos expression and onset of neuronal damage in CA3a following icv KA injection might be transiently delayed by icv NMDA preconditioning.

1. Introduction

Kainic acid (KA) is a cyclic analog of glutamic acid, which has been used to induce excitotoxic damage to neurons and glial cells, both in vivo and in vitro. The systemic administration of KA causes limbic seizures in rodents and it was, therefore, often employed as a model of human temporal epilepsy (Nadler, 1981, Olney, 1978, for review see Ben-Ari and Cossart, 2000). The seizures were followed by excitotoxic neurodegeneration in the hippocampus, piriform and

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entorhinal cortex, amygdala and dorsal thalamic nuclei. Such neurodegeneration included signs of morphological deterioration of nerve cell perikarya and their processes on the way to cell death. The excitotoxic neurodegeneration was accompanied by the expression of various genes, including immediate early genes (IEG) and stress protein genes (for review see Zagulska-Szymczak et al., 2001; Tang et al., 2002; Hatazaki et al., 2007). Comparing genomic responses after KA with those following ischemia, hemorrhage or hypoglycemia showed that all genes induced by KA were also activated by the latter insults (Tang et al., 2002).

Although systemic injection of KA induced robust expression of IEG protein c-Fos and c-Jun and heat shock protein Hsp70 in pyramidal neurons of hippocampal fields CA1 and CA3, their expression did not protect these neurons against ongoing neurodegeneration (Hashimoto et al., 1998; Yoneda et al., 1999). On the contrary, KA-induced neurodegeneration of hippocampal neurons could be alleviated to a variable degree by preceding treatment-preconditioning-with various drugs, like barbiturates, caspase inhibitors, antioxidants, adenosine agonist, K_{atp} channel openers, polyunsaturated fatty acids, etc. Recently, a considerable protection of hippocampal neurons against systemic KA administration was achieved by in vivo activation of NMDA subtype of glutamate receptors (Ogita et al., 2003). In their report, the effective preconditioning was seen in mice injected with NMDA 1 h to 24 h before KA. However, prolonged activation of NMDA receptors seemed to be harmful since intrahippocampal 4-aminopyridine perfusion caused hippocampal neuronal death supposedly due to excessive release of glutamate (Ayala and Tapia, 2005). These studies in vivo confirmed the findings about survival promoting or neurodegeneration inducing effects of NMDA receptor stimulation in vitro (Hardingham and Bading, 2003, Soriano et al., 2006, Hardingham, 2006).

Recently, the selective deletion of hippocampal *c-fos* gene enhanced KA-induced neurodegeneration by delaying brain derived neurotrophic factor (BDNF) expression in mutant as compared to wild type mice (Zhang et al., 2002; Dong et al., 2006). Calcium influx through NMDA receptor plays an important role in the regulation of c-AMP response element binding protein (CREB), a transcription factor, which is a potent activator of pro-survival BDNF expression (Hardingham et al., 2006). Furthermore, CREB is also participating in an in vivo expression of several IEG following NMDA receptor activation (Cole et al., 1989, Wisden et al., 1990).

Hippocampal changes induced by KA in vivo are influenced by way of its administration. The intracerebroventricular (icv) injection of sub microgram amounts of KA in the rat produced more restricted damage to hippocampal neurons as compared to systemic administration (Nadler et al., 1980, Wilde et al., 1994, Lee et al., 2002). Both neurochemical and morphological changes developed swiftly in the CA3 region while other hippocampal regions remained spared. The initial signs of IEG expression were already present by half an hour and typical cell damage was observed by 2–4 h in the CA3 region (Nadler et al., 1980; Zagulska-Szymczak et al., 2001). Thus, the icv KA administration seems to be more suitable for the study of acute stages of neurodegeneration, and hopefully, of the mechanism(s) of protective preconditioning. Following this line of reasoning, we attempted to elucidate the relation of c-Fos protein induction in CA3 neurons to their degeneration in the first 4 h after icv KA injection and the possibility of protecting them by inducing c-Fos with NMDA preconditioning.

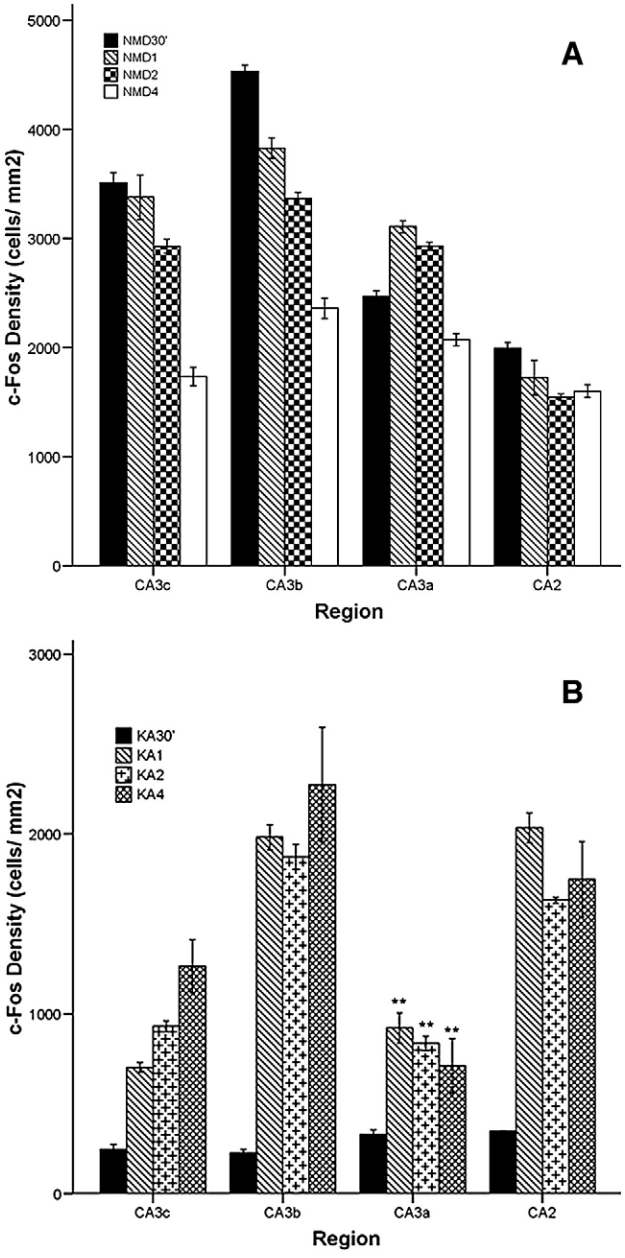

Fig. 1 – Time course of c-Fos induction in hippocampal CA2 and CA3 sub regions following icv NMDA or KA injections. (A) A steep increase of c-Fos immunostaining induced by icv NMDA (3 μg) at 30 min (NMD30′) and 1 h (NMD1) post injection in all regions was followed by a decline through 2 h (NMD2) and 4 h (NMD4), (n = 4 for each time interval). (B) In comparison with NMDA, KA-induced increase of c-Fos staining was slower at 30 min (KA30′). However, steady state levels of c-Fos in CA3b and CA2 contrasted with attenuated c-Fos expression in CA3a at 1 (KA1), 2 (KA2) and 4 (KA4) h post injection (n = 4 for each time interval).