Research report

Seizure-induced neuronal death is associated with induction of c-Jun N-terminal kinase and is dependent on genetic background

Paula Elyse Schauwecker*

Department of Cell and Neurobiology, University of Southern California, Keck School of Medicine, BMT 401, 1333 San Pablo Street, Los Angeles, CA 90033, USA

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Abstract

Previous studies have shown that expression of c-Jun protein, as well as the c-Jun amino-terminal kinase (JNK) group of mitogen-activated protein kinases, may play a critical role in the pathogenesis of glutamate neurotoxicity. In order to define the molecular cascade that leads to c-Jun activation following excitotoxic injury and delineate whether induction of protein synthesis is related to cell death signaling cascades or those changes associated with increased seizure activity, we examined the expression of JNK-1, as well as its substrate, c-Jun and N-terminal phosphorylated c-Jun following kainic acid (KA) administration in two strains of mice. In the present study, we assessed the immunohistochemical expression of these proteins at time points between 2 h and 7 days, in excitotoxic cell death-resistant (C57BL/6) and -susceptible (FVB/N) mouse strains that were systemically injected with saline or kainic acid. No strain-related differences in the immunohistochemical expression of any of the proteins were observed in intact control mice. However, following KA administration, the magnitude and period of induction of JNK-1 protein was associated with impending cell death, while increased phosphorylation of c-Jun protein was associated with resistance to cell death. In contrast, expression of c-Jun protein does not appear to be a reliable indicator of impending cell death, as it was expressed in resistant and vulnerable subfields in mice susceptible to kainate injury. These results provide the first evidence that JNK-1 expression may be involved in producing the neuronal cell death response following excitotoxin-induced injury. © 2000 Elsevier Science B.V. All rights reserved.

Theme: Disorders of the nervous system

Topic: Neurotoxicity

Keywords: Hippocampus; Immunocytochemistry; Excitotoxicity; Mouse strain

1. Introduction

Systemic administration of kainic acid (KA), a model of excitotoxicity, induces recurrent seizures and epileptiform activity in rodents. The seizures cause selective excitotoxic cell death of pyramidal neurons in the CA1 and CA3 subfields and dentate hilar neurons of the hippocampus, while sparing neurons in the CA2 subfield and dentate granule neurons [4,31,34,41]. Excitotoxic cell death is triggered by a massive release of glutamate, which activates glutamate receptors leading to dramatic increases in intracellular Ca$^{2+}$ [7]. The increased Ca$^{2+}$ levels initiate signaling cascades within susceptible neurons resulting in neuronal death. Although the mode of neuronal cell death induced by excitotoxins has been widely debated, previous studies have suggested that this type of cell death exhibits some of the characteristics of both necrosis and apoptosis, depending on the severity of the stimulation and the levels of intracellular free Ca$^{2+}$ [14,23,28,42].

While the molecular mechanisms of cell death are not well understood, excitotoxic cell death may result from the activation of intracellular signaling cascades that may be genetically determined. One of these signaling cascades, the c-Jun amino-terminal kinase (JNK)/SAPK pathway, is a component of signal transduction cascades that results in cell stress and death, and can be activated by a variety of cellular stresses, such as heat shock, osmotic imbalance, oxidative stress, and excitotoxicity [1,15,21,26,35,48]. The JNK/SAPK kinases are composed of three isoforms, JNK-1, JNK-2, and JNK-3, all of which have been proposed to
be important mediators of stress-activated signal transduction pathways [20].

JNKs are also important post-translational modifiers of c-Jun activity [3,11], in that they can increase the transcriptional activity of c-Jun by phosphorylation on serine 63 and serine 73, located within the amino terminal activation domain [8,22,37,43]. Increased c-Jun expression has been identified both in neuronal populations with the capacity to survive an excitotoxic insult [44], as well as those populations where selective neuronal degeneration occurs [9]. However, the association between expression of immediate early genes, such as c-Jun, and their putative role as cell death-effectors or -repressors following excitotoxic cell death remains unclear.

While the environmental and genetic cues leading to in vivo cell death of neurons are not well understood, our model of strain-related differences in susceptibility to excitotoxic cell death provides a means for differentiating between those molecular events that govern cell death and protection versus those that occur in response to cell stress. Previously, we had found that susceptibility to KA-induced cell death is strain-dependent, yet seizure activity is comparable between strains [39,40]. Thus, we were interested in determining how the genetic program in C57BL/6 mice (a representative resistant strain) is altered to allow protection against excitotoxic cell death. The present experiments were performed to evaluate the relationship between seizure induction, expression of the immediate early gene (c-Jun), phosphorylation of c-Jun, and its upstream regulator (JNK), and cell loss within the hippocampus in mouse strains resistant and susceptible to kainic acid-induced excitotoxicity.

In this article, we show that increased expression of JNK-1 occurred only in mice susceptible to excitotoxic cell death and was never solely associated with a cell stress response. In contrast, enhancement of c-Jun phosphorylation was observed only in those cells resistant to seizure-induced cell death or that survived the excitotoxic insult. Expression of c-Jun protein does not appear to be indicative of susceptibility or resistance to excitotoxic cell death as protracted expression of c-Jun protein was observed in both damaged and resistant cell populations in mice susceptible to excitotoxic cell death. These findings demonstrate that increased expression of JNK-1 is associated with KA-induced cell death and provides further support for the hypothesis that the JNK/SAPK cascade is involved in excitotoxic cell death.

2. Materials and methods

2.1. Animals

Adult male C57BL/6 (60-days-old) mice purchased from B&K (Fremont, CA) and adult male FVB/N mice, purchased from Jackson Laboratories (Bar Harbor, ME) served as subjects. All mice were housed individually on a 12-h light/dark schedule. Water and food were available ad libitum.

2.2. Drug administration

All experimental procedures were performed in accordance with approved institutional animal research protocols. Kainic acid was dissolved in isotonic saline (pH 7.3) and administered subcutaneously. Previous studies have defined seizure thresholds and revealed consistent seizures in both strains with a mortality rate of less than 25% at a dose of 30 mg/kg, s.c. [39]. Following KA administration, mice were monitored continuously for 3–4 h for the onset and extent of seizure activity. Seizures were rated according to a previously defined scale [38]: Stage 1, immobility; Stage 2, forelimb and/or tail extension, rigid posture; Stage 3, repetitive movements, head bobbing; Stage 4, rearing and falling; Stage 5, continuous rearing and falling; and Stage 6, severe tonic-clonic seizures. All mice included in the study exhibited at least 1 h of continuous Stage 5 seizures.

2.3. Histological staining and immunohistochemistry

Following survival times of 2, 6, 24, 48, 72, or 168 h (n=5 or 6 per time point), mice received an overdose of sodium pentobarbital and were perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) following saline or KA administration. Brains were immediately removed and post-fixed overnight in the same fixative before being cryoprotected in 30% sucrose in 0.1 M phosphate buffer (pH 7.3). Horizontal sections (35 μm) were cut on a sliding microtome (Leica, Deerfield, IL) and incubated in 0.1 M phosphate buffer (pH 7.4) prior to immunohistochemical staining. For histological assessment, every sixth section was stained with cresyl violet to determine neuronal cell loss and the general histological features of the tissue. For immunohistochemistry, every sixth section was rinsed in 0.1 M phosphate buffer (pH 7.4) three times for 15 min each, incubated for 2 h on a rotary shaker in a blocking buffer containing 0.5% Triton-X and 5% normal goat serum in 0.1 M phosphate buffer (pH 7.4). Sections were then incubated overnight at 4°C with one of the following polyclonal antibodies: c-Jun (1:500; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), JNK-1 (1:500; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), or phospho-c-Jun (Ser73; 1:1000; Upstate Biotechnology, NY) in the presence of 5% normal goat serum in 0.1 M phosphate buffer (pH 7.4). Sections were rinsed in 0.1 M phosphate buffer (pH 7.4), incubated for 2 h at room temperature in goat anti-rabbit biotinylated IgG (1:200 in 0.1 M phosphate buffer containing 5% normal goat serum), and then for 1 h at room temperature in 1% ABC reagent (Vector Laboratories, Burlingame, CA). Subsequent antibody visualization was carried out using 3,3’-diaminobenzidine (DAB; Sigma, St. Louis, MO) as
the chromagen with 0.01% H$_2$O$_2$ as peroxidase substrate for 2–5 min in imidazole buffer containing 2.5% nickel ammonium sulfate. Immunostained sections were then mounted onto gelatiniized slides, dehydrated in graded alcohols, cleared in xylene and coverslipped. Immunocytochemical controls consisted of the same reaction procedures described above in the absence of primary antibody.

2.4. Assessment of neuronal damage

Horizontal sections at the level of the dorsal and ventral hippocampus were examined for cell loss in both strains of mice. Briefly, the number of degenerating neurons in both the right and left hippocampus from every sixth section (240 μm separation distance) was counted and averaged into single values for each animal. The number of degenerating neurons in three brain regions (CA3, CA1, dentate hilus) were visually estimated and a histological damage score was assigned on a 0–3 grading scale: grade 0, none; grade 1.0, mild (10–25%); grade 2.0, moderate (46–54%); and grade 3.0, severe (>75%), according to a previously defined scale [16–18]. All grading was performed blindly by an observer who was naïve to strain, time point, and treatment (KA vs. saline injection). Since histological damage scores were normally distributed, we were able to use standard parametric methods of data analysis. Thus, in order to determine if differences in histological scores existed among the groups of mice, results were assessed statistically by one-way ANOVA using the computer program, SigmaStat (Jandel Scientific, San Rafael, CA), and intergroup differences were analyzed by Newman–Keuls post-hoc test.

2.5. Quantitative analysis of immunohistochemistry

To assess the distribution and expression of c-Jun, phospho-c-Jun, and JNK-1 immunoreactive cells, sections stained for immunoreactivity were examined under bright field illumination with a Nikon microscope and analyzed with the Image-Pro image analysis system (Image-Pro Plus, Silverspring, MD) in a blinded fashion. Images were digitized and the threshold was adjusted manually so that immunostained cells were above threshold and unstained areas were below threshold. The percentage of area occupied by stained cells (proportional area) in hippocampal regions (CA1, CA3, and dentate gyrus) was compared between C57BL/6 and FVB/N mice by performing all measurements with a rectangular shaped box (0.3×0.05 mm$^2$). Three measurements within each hippocampal region were taken from at least three to five sections from each animal. Measurements were expressed as ratios of ipsilateral values/intact values in order to control for variations in immunostaining. Results were expressed as mean±S.E.M. Statistical analyses were performed using a one-way ANOVA and Newman–Keuls post-hoc test using the statistical software program, SigmaStat (Jandel Scientific, San Rafael, CA). Differences were considered significant when $P<0.05$. Comparisons were always made between control C57BL/6 and FVB/N mice.

2.6. Protein extraction, gel electrophoresis and Western blotting

For these analyses, fresh brains were rapidly removed from the skulls. Hippocampi from C57BL/6 and FVB/N mice were micro dissected from intact mice and from mice 2, 24, and 72 h following KA administration ($n=6$ per each time point and per each strain) and stored at −70°C until use. For gel electrophoresis and Western blotting, hippocampi were transferred to ice-cold lysis buffer (Tris-buffered saline, pH 7.4 containing 1% Triton X-100, 10% glycerol, 0.5 mM sodium orthovanadate, and 1 mM phenylmethylsulfonyl-fluoride) and the protein content of hippocampi was transferred to ice-cold lysis buffer (Tris-buffered saline, pH 7.4 containing 1% Triton X-100, 10% glycerol, 0.5 mM sodium orthovanadate, and 1 mM phenylmethylsulfonyl-fluoride) and the protein content of hippocampal tissue was resolved on 10% S.D.S-polyacrylamide gels (Invitrogen, Carlsbad, CA) and blotted onto Protran transfer membranes (Schleicher and Schuell, Keene, NH). Membranes were blocked for 1 h at room temperature with 5% non-fat dried milk in phosphate-buffered saline/Tween-20 (0.1% Tween-20) and probed with a mouse anti-human JNK monoclonal antibody at 1:2000 that recognizes both JNK-1 (JNK-46) and JNK-2 (JNK-54; Santa Cruz Biotechnology, Santa Cruz, CA). The antigen–antibody complexes were visualized with the enhanced chemiluminescence ECL system (Amersham, Arlington Heights, IL) after incubating the blot with HRP-

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Seizure parameters (% of mice)</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
<th>Stage 5</th>
<th>Duration of seizures (h)</th>
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<tr>
<td>C57BL/6 ($n=56$)</td>
<td>100±0</td>
<td>100±0</td>
<td>100±0</td>
<td>100±0</td>
<td>87.50±5.76$^a$</td>
<td>1.18±0.11$^a$</td>
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<tr>
<td>FVB/N ($n=47$)</td>
<td>100±0</td>
<td>100±0</td>
<td>100±0</td>
<td>100±0</td>
<td>97.82±4.00$^a$</td>
<td>1.39±0.09</td>
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$^a$ KA induced a similar level of seizure duration and stage irrespective of mouse strain. Data are represented as mean±S.E.M.

$^a$ Statistically significant strain difference: $F=3.909; P=0.051$.

$^a$ Statistically non-significant strain difference: $F=1.989; P=0.161$.
Fig. 1. Photomicrographs of horizontal sections of the hippocampus showing the time course of cell loss following kainic acid administration in C57BL/6 and FVB/N mice. Mice of both strains were injected with saline (top panels) or systemically injected with kainic acid. Cryosections were stained with cresyl violet. Note the extensive cell loss in the CA3 subfield (arrows) and within the hilus in FVB/N mice following kainate administration. CA3, CA3 pyramidal cell layer; CA1, CA1 pyramidal cell layer; H, hilus. Scale bar=750 μm.
conjugated (horseradish peroxidase-conjugated) goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA), followed by apposition of the membranes with autoradiographic film (Hyperfilm ECL, Amersham, Arlington Heights, IL).

3. Results

3.1. Seizure effects following systemic administration of KA

Following systemic administration of KA, all mice (n = 103) underwent a series of behavioral seizure stages as previously described [39,40]. Within 10 min of injection, mice assumed a catatonic phase and were immobile. Within 20–30 min post-injection, mice exhibited forelimb clonus and hindlimb clonus, and within 45 min post-injection, all mice were rearing and exhibited tonic-clonic seizures. Seizures lasted on average 1.3 h and no significant strain-dependent effects were found with regard to the duration of seizures (Table 1). Table 1 summarizes the percentage of animals from each strain achieving each seizure stage (1 through 5) and the duration of Stage 5 seizures for each of the strains. A nearly significant difference in the percentage of animals achieving Stage 5 seizures was observed between C57BL/6 and FVB/N mice. However, it is important to note that, regardless of strain, nearly 90% of all animals administered KA exhibited Stage 5 seizures.

3.2. Time course of hippocampal cell loss following systemic administration of KA

In accordance with previous studies [39,40], administration of KA led to the degeneration and loss of CA3 pyramidal neurons and hilar neurons, and occasionally CA1 pyramidal neurons in FVB/N (susceptible) mice, while no cell loss was detectable at any time following KA administration in C57BL/6 mice (resistant; Figs. 1 and 2). In accordance with previous studies [32,33,45], cells within the dentate granule cell layer and area CA2 of Ammon’s horn were resistant to KA-induced damage in susceptible mice. Within 2 h following administration of KA, a small degree of cell loss was observed within the hilus and area CA3b of Ammon’s horn (Fig. 2). However, by 24 h post-KA administration, significant cell loss was observed in the dentate hilus, area CA3, and area CA1. At this time point, increased argyrophilic deposits were present throughout the stratum oriens and stratum pyramidale of the CA3 and CA1 subfields and within the dentate hilus. Cell loss was still evident at 7 days post-KA administration (Figs. 1 and 2).
3.3. Effect of systemic KA administration on expression of JNK-1

Previous studies have suggested that the elevated expression of c-Jun and JNK is closely linked to neuronal death after glutamate-induced excitotoxicity [21,25,30,49]. In the present study, we examined the expression of JNK-1 at the protein level using immunocytochemical and immunoblotting techniques in the brains of saline- and KA-treated resistant (C57BL/6) and susceptible (FVB/N) mice, focusing on the hippocampal region, which is particularly susceptible to KA-induced cell death. Using an anti-JNK-1 antiserum, which specifically reacts with JNK1 p46 and JNK2 p54 of mouse, rat and human origin (Santa

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**Fig. 3.** Low-power immunoreactive images of JNK-1 protein in the hippocampus after saline injection (A, B) and 72 h following KA administration in C57BL/6 and FVB/N mice (C, D). High-power photomicrographs of JNK-1 immunoreactivity in the dentate hilus (E, F) and area CA3 (G, H) 72 h following KA administration. Note the induction of JNK-1 within remaining neurons in area CA3 (H) in FVB/N mice and the absence of JNK-1 from dentate hilar cells (F). Scale bar=750 μm (A, B, C, D); Scale bar=350 μm (E, F, G, H); Scale bar=150 μm (insets of E, F, G, H).
Cruz Biotechnology, Santa Cruz, CA), we found that normal control hippocampus from either strain of mouse showed moderate cytoplasmic and nuclear immunoreactivity throughout all hippocampal cell areas (dentate granule cells, dentate hilar cells, area CA3, area CA2, and area CA1; Fig. 3). Following administration of KA, increased JNK-1 immunoreactivity began to be detectable as early as 2 h post-KA administration in CA3 and CA1 pyramidal neurons and in dentate hilar neurons of FVB/N mice (susceptible) and became maximal between 72 and 168 h post-administration (Figs. 3 and 4). As shown in Fig. 3, JNK-1 reaction product is present within both the cytoplasm and nucleus of hippocampal neurons at early time points (panels A, B), and it appears that at 72 h following KA administration induction in the remaining CA3 pyramidal neurons may have a more nuclear localization (panel H), although this was difficult to discern. However, based on our temporal analysis of cell loss following KA administration (Figs. 1 and 2), these data indicate that increased JNK-1 immunoreactivity precedes neuronal loss in areas CA3, CA1, and the dentate hilus. Although increased immunoreactivity was observed in resistant dentate granule cells of FVB/N mice between 48 and 72 h following KA administration (Fig. 4), it was never observed in resistant CA2 pyramidal cells. In contrast, increased expression of JNK-1 protein was never observed in resistant (C57BL/6) mice at any time point following the administration of KA (Figs. 3 and 4).

Western analysis conducted on samples collected from intact C57BL/6 and FVB/N mice and at 2, 24 and 72 h following KA administration confirmed the specificity of the JNK antibody that recognized both JNK-1 (46 kDa) and JNK-2 (54 kDa) isoforms (Fig. 5). Immunoblotting of protein extracts revealed that the JNK-1 isoform was significantly induced in FVB/N mice at 2, 24 and 72 h following KA administration, while no significant difference in the mean optical density of the JNK-1 or JNK-2 isoform was observed in C57BL/6 mice (Fig. 5). These results are consistent with the semi-quantitative analysis of hippocampal immunostaining in C57BL/6 and FVB/N mice following KA administration (Fig. 4).

3.4. Effect of systemic KA administration on expression of c-Jun

Basal levels of specific nuclear c-Jun staining were found only in neurons of the dentate granule cell layer. No significant strain differences were observed in any brain regions in saline-injected animals. In those mice resistant to KA-induced cell death (C57BL/6), c-Jun immunoreactivity showed a prolonged increase in neurons of the dentate granule cell layer alone following KA administration, while only a transient induction was observed in pyramidal cells (Figs. 6 and 7). In contrast, mice susceptible to KA-induced cell death (FVB/N), displayed prolonged increases in c-Jun immunoreactivity especially in those remaining neurons within the pyramidal cell layer (Fig. 7). It is important to note that although c-Jun protein was transiently and modestly increased in less vulnerable

![Graphs](image-url)
cells, such as dentate granule cells, at early time points after KA administration in FVB/N mice, a greater and prolonged increase in c-Jun immunoreactivity was found in those neurons destined to die in FVB/N mice at all time points examined (Figs. 6 and 7).

3.5. Effect of systemic KA administration on expression of phospho-c-Jun

Using the antibody against human phospho-c-Jun, which visualizes N-terminally phosphorylated c-Jun at its serine 73 residue in adult mouse brain, we found that control hippocampus from both mouse strains showed basal levels of phospho-c-Jun staining in dentate granule neurons of the dentate gyrus. However, following KA administration, increased expression of phospho-c-Jun was apparent within all neuronal layers of the hippocampus proper in C57BL/6 mice (representative resistant strain; Figs. 8 and 9) and persisted for up to 7 days following KA administration. In contrast, following KA administration to FVB/N mice (representative susceptible strain), phospho-c-Jun immunoreactivity was increased dramatically within neurons of the dentate granule cell layer as early as 2 h post-administration and persisted for up to 7 days post-administration (Fig. 9). Increases in phospho-c-Jun immunoreactivity were also observed in neurons within the CA3 and CA1 pyramidal cell layers at early time points, but within 24 h following KA administration, when significant cell loss was observed (Figs. 1 and 2), immunoreactivity was only elevated in those remaining neurons in areas CA3 and CA1 (Figs. 8 and 9).

4. Discussion

Studies investigating the genetic regulation of cell death have provided insight into the numerous genes and proteins that act either to protect or to induce cell death in a variety of cell types. In fact, a number of genes may be modulators of neuronal cell death resulting from seizure-induced injury [28,39,46,50]. Thus, the aim of this study was to examine the relationship between excitotoxic cell death and involvement of the JNK/SAPK signaling pathway following KA administration in mice both resistant and susceptible to excitotoxin-induced injury. We took advantage of genetic differences in susceptibility to excitotoxic cell death to provide a means for dissociating those genes induced by increases in KA-induced seizure activity from those involved in KA-induced hippocampal cell loss. The principal finding of the present study is that immunoreactivity for JNK-1 is only expressed in hippocampal neurons from mice susceptible to KA-induced injury (FVB/N) following KA administration and is not induced in mice resistant to KA-induced injury (C57BL/6). These results support the concept that JNK-1 may be an inducible cell death effector in the brain following KA administration.

Historically, KA-induced seizures have been associated with degenerating neurons in the hippocampus, particularly within the dentate hilar region and in the CA1, CA3 subfields of the hippocampus [32,33,45]. Previous studies have demonstrated that increases in seizure activity result in the activation of specific genetic programs that may be associated with subsequent cell death. Activation of the JNK/SAPK cascade has been shown to be associated with induction of various cellular stresses, such as heat shock, osmotic imbalance, oxidative stress, and excitotoxicity [1,15,21,26,35,48]. However, the mechanism by which stressful stimuli result in induction of JNK remains to be determined. In order to examine the cellular mechanisms that may regulate KA-induced cell death, the induction of the JNK/SAPK cascade was examined in two representative susceptible and resistant strains of mice. JNK-1 was only induced in mice susceptible to KA-induced cell death (FVB/N). These results are in agreement with previous studies that have examined induction of JNK-1 in rats following KA administration [29]. Resistant mice (C57BL/6) show no induction of JNK-1 protein in any region.
Fig. 6. Time course of c-Jun immunoreactivity in the hippocampus of C57BL/6 and FVB/N mice following saline injection (A, B) and 6 (C, D) and 72 h (E, F), following KA administration, respectively. In control mice of either strain, only dentate granule neurons contain immunoreactive product. Following KA administration, both strains show increased immunoreactivity in dentate granule neurons as well as pyramidal neurons in areas CA3 and CA1 at early time points (a, b, c, d). However, only FVB/N mice show a prolonged induction of c-Jun at later time points within the remaining pyramidal neurons of area CA3 (h) and in dentate granule neurons (g). Scale bar=750 μm (A, B, C, D, E, F); Scale bar=150 μm (a, b, c, d, e, f, g, h).

following KA administration, lending further support for a role for JNK-1 in excitotoxic cell death. However, the extent to which the various JNK isoforms exert different functions must still be clarified.

c-Jun is a transcription factor whose activity is regulated by N-terminal phosphorylation at serine 63 and serine 73 through all isoforms of JNK [8,27]. While some studies have suggested that c-Jun is an essential substrate for
unclear whether induction of c-Jun precedes cell death and whether increased c-Jun immunoreactivity occurs only in those cells committed to death. Here, we show that while prolonged induction of c-Jun occurred only in mice susceptible to KA-induced cell death (FVB/N), increased c-Jun protein was present in susceptible and resistant hippocampal neuronal fields. In addition, a transient increase in c-Jun protein was also observed throughout the hippocampus in resistant mice. These results suggest that although temporal expression of c-Jun protein can be correlated with either activation of seizure cascades or impending cell death, c-Jun protein is not an accurate predictor of excitotoxic cell death.

Previous studies have suggested a role for c-Jun phosphorylation in certain forms of cell death [5,11,47], and suggested that activation of the JNK isoforms and c-Jun phosphorylation may play a role in the elicitation of a neuronal death cascade [10,12,48,49]. In the present study, we detected serine-73 phosphorylated c-Jun throughout the hippocampus of C57BL/6 (resistant) mice following KA administration. In contrast, following KA administration, serine-73 phosphorylated c-Jun was only induced in those cells resistant to excitotoxic cell death (dentate granule neurons) or in those cells that survived the insult (area CA3). Thus, in contrast to our observations with JNK-1, the temporospatial distribution of serine-73 phospho-c-Jun correlated with selective resistance to seizure-induced cell death. Thus, our studies demonstrate incongruence between the spatial expression of JNK-1 and phospho-c-Jun protein. Although the reason for this discrepancy remains to be elucidated, it is possible that a molecule other than JNK-1 may be responsible for phosphorylating c-Jun. Nevertheless, results presented in this paper suggest that expression of serine-73 phosphorylated c-Jun may be related to resistance to seizure-induced cell death and that activation of JNK-1 does not necessarily result in N-terminal serine-73 phosphorylation of c-Jun.

In conclusion, our results demonstrate that expression of JNK-1 is induced by KA-induced cell death and provides further support for the hypothesis that the JNK/SAPK cascade is involved in excitotoxic cell death. Further experiments are necessary to determine the potential role of JNK-1 in KA-induced cell death. In contrast, N-terminal phosphorylation of c-Jun appears to be involved in resistance to seizure-induced cell death in the adult mouse brain, while c-Jun expression is not an accurate predictor of excitotoxic cell death. Although the molecular mechanisms by which cell death is induced and regulated remain unclear, our findings suggest that differential regulation of genes involved in cell death signaling cascades may contribute to the selective vulnerability of hippocampal neurons following KA administration. It remains to be determined which cellular signaling cascades might contribute to the propagation of cell death and which are merely part of the neuronal stress response.
Fig. 8. Immunodetection of phospho-c-Jun in the hippocampus of C57BL/6 and FVB/N mice following saline injection (A, B) and 72 (C, D) h following KA administration. High-power photomicrographs of phospho-c-Jun immunoreactivity in the dentate gyrus (E, F) and area CA3 (G, H) 72 h following KA administration. Note the induction of phospho-c-Jun in neurons within the dentate granule cell layer (E, F) of both strains. Although increased immunoreactivity is observed throughout the CA3 pyramidal cell layer of C57BL/6 mice (G), increased phospho-c-Jun is only present within remaining neurons in area CA3 of FVB/N mice (H). Scale bar=750 μm (A, B, C, D); Scale bar=150 μm (E, F, G, H).

our model of genetic differences in susceptibility to excitotoxic cell death proves a useful tool for dissecting the molecular mechanisms related to a cell death cascade and delineating them from those changes associated with seizure activity.

Acknowledgements

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Figure 9. Quantitative temporal analysis of immunoreactivity for phospho-c-Jun in hippocampal cell fields in C57BL/6 (resistant) and FVB/N (susceptible) mice following KA administration. Comparison of the temporal induction of phospho-c-Jun immunoreactivity between mouse strains revealed statistically significant differences between 6 and 168 h in areas CA1 and CA3 (F=15.37; P<0.001, Newman–Keuls test). Significant induction of phospho-c-Jun immunoreactivity was observed in dentate granule neurons at all time points following KA administration regardless of strain. Data represent the mean±S.E.M. of five to six mice/time point for each strain. * P<0.05 as compared to intact mice of either the C57BL/6 or FVB/N strain.

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References


