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Research report

Intracerebral injection of caspase-3 inhibitor prevents neuronal apoptosis after kainic acid-evoked status epilepticus

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Abstract

In the aftermath of prolonged continuous seizure activity (status epilepticus, SE), neuronal cell death occurs in the brain regions through which the seizure propagates. Recent studies have implicated apoptotic processes in this seizure-related injury. Because activation of caspase-3-like cysteine proteases plays a crucial role in mammalian neuronal apoptosis, we explored the possibility that activation of caspase-3 is involved in the neuronal apoptotic cell death that occurs in rat brain following SE induced by systemic kainic acid. Caspase-3 activity was determined immunocytochemically using CM1 antibodies specific for catalytically active subunit (p17) of the enzyme. We found an induction of caspase-3 activity in rhinal cortex and amygdala at 24 h after SE. To determine whether activation of caspase-3-like proteases is a *necessary* component of the injury process, we delivered a caspase-3 inhibitor, z-DEVD-fmk, into the lateral ventricle prior to, and following SE. z-DEVD-fmk treatment substantially attenuated apoptotic cell death after SE, both in hippocampus and rhinal cortex, as evaluated by analysis of internucleosomal DNA fragmentation and neuronal nuclear morphology. Our findings implicate caspase-3 cysteine protease in the neurodegenerative response to SE and suggest that this degeneration can be attenuated by inhibition of caspase-3-like enzyme activity.

Keywords: Epilepsy; Limbic system; Rhinal cortex; Caspase-3; Neuronal apoptosis; DNA fragmentation

1. Introduction

Prolonged continuous seizure activity, as occurs during status epilepticus (SE), triggers neuronal cell death in the brain regions through which the seizure propagates. Experimental SE evoked by the systemic administration of kainic acid is associated with neuronal damage preferentially localized to limbic areas, including hippocampal CA subfields and rhinal cortex [37,38].

There is reason to suspect that programmed cell death (PCD) is responsible for a significant component of the neural degeneration that occurs in the aftermath of prolonged seizures. Molecular and histopathological markers of PCD, some of which characterize apoptosis, have been reported as sequelae of SE evoked by either kainic acid or pilocarpine [13–15,19,20,32–34,44].

Apoptotic PCD depends to a significant extent on the activation of cysteine proteases. The *ced-3* gene, initially identified in the nematode, *Caernorhabditis elegans*, provided the first evidence for an essential role of cysteine proteases in promoting apoptosis [45]. Multiple mammalian homologues of Ced-3, or ''caspases'', have since been identified and fall into two distinct subclasses: caspase-1-like and caspase-3-like proteases. Among those related to caspase-1 are caspases 4, and 5 [8,18,28]. Those similar to caspase-3 include caspases 2, and 6-10 [6,9–12,23,29,42].

In mammalian systems, a crucial role for caspases in neuronal apoptosis has been documented through the use of specific inhibitors of cystein proteases in cell cultures [4,5,7,14,26,36,40]. Moreover, caspases have been implicated in the pathogenesis associated with several models of brain damage, including ischemia [2,3], traumatic brain injury [43], and epilepsy [16]. In particular, induction of caspase-3 mRNA has been reported in rat hippocampal neurons following systemic administration of kainic acid [16], after transient global ischemia [2,16], and in response to fluid percussion-induced traumatic brain injury [43]. Furthermore, inhibitors of caspase-1-like or caspase-3-like cysteine proteases proved neuroprotective against ischemic

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insults in vivo [2,3] and against ischemic and excitotoxic treatments in vitro [17].

Post-translational activation of caspases requires proteolytic cleavage of the precursor protein. In the case of caspase-3, two subunits (p17 and p12) are generated, the larger of which contains the catalytic site [10]. This process (and the resulting generation of proteolytic fragments) leads to enzyme activation and can therefore be used as a reliable and sensitive indicator of caspase activation. In the present study, we examined apoptosis and proteolytic cleavage of caspase-3 in rat hippocampus and rhinal cortex following kainic acid-evoked SE of durations sufficient to cause mild to moderate degrees of neuronal injury. To determine whether caspase-3 activation is a *necessary* component of SE-induced apoptosis, we evaluated the effect of a specific caspase-3 inhibitor, z-DEVD-fmk, applied by intracerebroventricular microinjection (according to a protocol established by Yakovlev et al. [43]), on the extent of apoptotic cell death following SE. Our findings indicate that caspase-3-like cysteine protease is a necessary component of the neurodegenerative response to prolonged seizure activity.

2. Materials and methods

2.1. Animals

Sprague–Dawley male rats weighing 175–200 g (Harlan Sprague Dawley, Indianapolis, IN) were used. Rats were maintained four per cage in a temperature-controlled room with a 12-h light cycle. Food and water were provided ad libitum. All experimental protocols used were in compliance with AALAC standards and were approved by the Georgetown University Animal Care and Use Committee.

2.2. Surgical procedures

Animals were anesthetized with Equithesin (3 mg/kg) and placed in a Kopf stereotaxic apparatus. A stainless steel guide cannula (0.71 mm external diameter, 3 mm length) aimed at the left lateral ventricle was placed through a hole drilled in the skull and secured to the scull with dental acrylic and jeweler's screws. A stainless steel stylet (0.36 mm external diameter) was inserted into the guide cannula and kept in place prior to, and in between, injections. Surgery was performed on the rats at least 3 days before experiments were initiated.

2.3. Treatment groups

Rats were randomly assigned to vehicle-treated control or z-DEVD-fmk groups ($n = 4$ for each group). A specific caspase-3 tetrapeptide inhibitor, z-DEVD-fmk (*N*-benzyloxycarbonyl-Asp(OMe)-Glu(OMe)-Val-Asp(OMe)-fluoromethylketone), 240 pmol, was infused into the left lateral ventrical in a volume of 5 μl. The infusion cannula, which protruded 1 mm below the indwelling guide cannula, was connected by polyethylene tubing to a 10 μl Hamilton syringe mounted in a Sage infusion pump. The treatment regimen we selected was identical to that used by Yakovlev et al. [43] for the prevention of neuronal death after traumatic brain injury. The treatment was given 30 min before kainic acid injection, and again at 4 and 20 h after the onset of SE (which occurred between 45 and 90 min after kainic acid injection, see below). Vehicle-treated control animals were infused at the same time points (before and after SE) with 5 μl of vehicle alone (50% DMSO). Animals were sacrificed by decapitation at 6 h after the last intraventricular injection (i.e., 24 h after termination of SE). Brains were either quickly dissected (for DNA isolation) or frozen for subsequent cryostat sectioning (for immunohistochemistry). The basis for selecting the 24-h time point (after SE termination) for measurements of caspase-3 activation and apoptosis included: (1) The fact that following kainic acid, DNA fragmentation in rhinal cortex and hippocampus has an onset between 12 and 24 h (with continued rise in fragmentation for up to 48 h or longer) [unpublished observations]; 24 h is the earliest time at which all animals exhibit consistent robust DNA fragmentation (a marker of irreversible stage of apoptosis); (2) Caspase-3 activation is known to precede the triggering of DNA fragmentation [31,39]. By choosing the earliest time point when fragmentation was robust, we increased our chances of detecting *both* caspase-3 activation *and* DNA fragmentation, thus allowing us to evaluate the protective effect of z-DEVD-fmk with maximum sensitivity.

In addition, two separate groups of saline-treated controls (see Induction of SE) and SE-treated rats ($n = 3$ for each group) were used for caspase-3 activity measurements.

2.4. Induction of SE

To induce SE, kainic acid (Sigma) was administered i.p. in a dose of 10–15 mg/kg, titrated as necessary for each group of animals to ensure that continuous SE developed within 90 min of injection. SE was allowed to continue for 2 h, after which it was terminated by an i.p. injection of 30 mg/kg diazepam (Elkins-Sinn, Cherry Hill, NJ). SE was defined as continuous seizure activity (facial and forelimb clonus, head bobbing, and facial twitching) uninterrupted by normal behavior. The animals were observed closely for at least 2 h following seizure termination to verify seizure cessation. We have previously established (in studies with EEG recordings) that in diazepam-treated rats, signs of mild head twitching and/or forelimb twitching are indicative of resumption of electrographic seizure activity. Consequently, additional doses of diazepam (10 mg/kg) were given, as needed, to ensure that all signs of seizure activity (including subtle twitching) were pre-

vented. Control (seizure naïve) rats received i.p. injection of saline (''saline control treatment'') instead of kainic acid, and were given diazepam (30 mg/kg i.p.) 3 h later.

2.5. Immunocytochemistry

2.5.1. Perfusion / fixation

For immunocytochemical detection of caspase activation, perfusion/fixation was performed. After completion of each experiment, animals were given an i.p. injection of pentobarbital (150 mg/kg) to induce deep anesthesia as assessed by loss of the ''toe-pinch reflex''. Fixation was then initiated by perfusion with a rinsing solution (phosphate-buffered saline, PBS, pH 7.4, Gibco). This was followed by perfusion with 4% formaldehyde in PBS (pH 7.4). Brains were then removed from the skull, soaked overnight in the same buffer, and then transferred to a formol-saline solution containing 20% sucrose for an additional 24 h.

2.5.2. Detection of caspase-3 activation

Caspase-3 activation was determined using CM1 rabbit polyclonal antibodies (IDUN Pharmaceuticals, La Jolla, CA) which specifically recognize the fragment (17 kDa) containing the catalytic site of the enzyme [21,41]. Procedures followed those recommended by the company, as previously described [41]. Briefly, coronal cryosections (10 μm) from perfusion-fixed brains were rinsed twice in PBS and incubated for 30 min in PBS containing 3% hydrogen peroxide to inhibit endogenous peroxidase activity. Sections were then incubated for 1 h at room temperature in blocking buffer consisting of 2% bovine serum albumin (Sigma), 0.2% non-fat milk powder, 2% normal goat serum (Sigma) and 0.8% Triton X-100 in PBS, and then overnight with primary antibodies (1:2000) at 4°C. Sections were washed three times in wash buffer (PBS containing 0.2% Tween-20), incubated with biotinylated goat anti-rabbit antibodies, and then incubated with avidin-horseradish peroxidase (HRP) conjugate (Vector Laboratories, Burlingame, CA), according to the manufacturer's recommendations for the Vecstain ABC kit. Active caspase-3-positive cells were visualized using chromogenic HRP substrate from the AEC kit (Vector Laboratories) as recommended by the manufacturer. To control for reaction specificity and residual endogenous peroxidase activity, adjacent brain sections were treated as described above with the omission of either CM1 antibody or goat anti-rabbit antibodies. Sections were counterstained with Hematoxylin (Vector Laboratories) to visualize nuclei and then examined under a microscope.

2.5.3. Double labeling for Hoechst 33258 and Texas Ted / NeuN

Immunohistochemical detection of apoptotic neurons (double labeling) was performed according to the procedures described previously [43]. Coronal cryosections (10 μm) containing ventral hippocampus were thaw-mounted onto Superfrost Plus slides (Fisher Scientific). The sections were immersion fixed for 5 min in 10% buffered formalin (pH 7.1), rinsed twice in PBS and then permeabilized by a 15 min immersion in PBS containing 0.1% saponin (Calbiochem). For identification of neurons, sections were incubated in a PBS buffer containing mouse antibodies to rat neuronal nuclear protein (NeuN, 1:100; Chemicon, Temecula, CA), 1% bovine serum albumin, and 1% normal goat serum (Sigma). After 20 h, the sections were washed in PBS and incubated for 1 h with fluorescent (Texas Red) goat anti-mouse IgG (1:100; Sigma) and then washed 3 × in PBS. All sections were then incubated for 1 min with a 1:5000 dilution of 10 mg/ml bis-benzimide (Hoechst 33258; Sigma) to resolve nuclear morphology, mounted in Citifluor (Ted Pella, Redding, CA), and examined under a fluorescent microscope with excitation/emission wavelengths of 345/425 (Hoechst) and 590/615 (Texas Red).

2.6. Analysis of DNA fragmentation

Apoptotic DNA was isolated and labeled as previously described [43], with minor modifications. Briefly, genomic DNA was extracted from rhinal cortex and hippocampus in 7 M guanidine hydrochloride. Extracts were mixed with 1 ml of Wizard Maxiprep Resin (Promega) and the suspension was pelleted by centrifugation. The pellets were resuspended in 1 ml of washing solution (90 mM NaCl, 9 mM Tris–HCl, pH7.4, 2.25 mM EDTA, and 55% ethanol), and drawn by vacuum through Wizard Midicolumns (Promega). Columns were washed with 6 ml of washing solution and vacuum dried. DNA was eluted with 50 μl of water. Residual RNA was removed by incubation with 1 μg of RNase A at 37°C for 30 min. DNA (100 ng) was added to each 20 μl of labeling mixture comprised of 10 mM Tris–HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100, 10 mM $MgCl_2$, 2 μCi [α-^{32}P]dATP (3,000 Ci/mmol, Amersham) and 1 U *Taq* DNA polymerase (Perkin-Elmer). Reactions were incubated at 72°C for 20 min and terminated by the addition of the gel loading buffer. Samples were loaded onto a 1.3% agarose gel and electrophoresed at 4 V/cm. Labeled DNA fragments were visualized by autoradiography of the dried gel. Multiple autoradiographic exposure durations were used to determine and ensure that visualized DNA fragments were in the linear range of optical density. Optical density of the nucleosomal dimer areas on the autoradiograph was measured using an AlphaImager 2000 image capturing computer (Alpha Innotech, San Leandro, CA) and image analyzing software (Scion Image 3b, Scion, Frederick, MD).

A two-way ANOVA with a post-hoc Fishers test was used for statistical analysis of the DNA fragmentation detected in hippocampus and rhinal cortex in the different treatment groups. The following groups of animals and

samples were compared: (a) vehicle-treated control group was compared with SE or SE + zDEVD-fmk treated groups; (b) SE-treated group was compared with SE + z-DEVD-fmk treated group; and (c) samples from hemisphere ipsilateral to the z-DEVD-fmk infusion site were compared to samples from contralateral side within the same treatment group.

3. Results

3.1. Induction of caspase-3 activity by SE

The induction of caspase-3 activity after SE was examined immunohistochemically using CM1 antibodies [41]. These antibodies selectively recognize the active (17 kDa) catalytic subunit of the enzyme, which is released by the proteolytic digestion of an inactive precursor protein. CM1 antibody staining of brain sections obtained from rats at 24 h after a 2-h period of kainic acid-induced SE revealed cells strongly positive for the active subunit of caspase-3 localized throughout rhinal cortex (Fig. 1d,d*). In addition, a modest level of immunoreactivity was seen in the medial and lateral mammillary body. No positive cells were detected in hippocampus (Fig. 1b), mediodorsal thalamus, parietal cortex or striatum (not shown) of the same animals. Rhinal cortex and hippocampus in brain sections from animals that did not experience SE, contained no cells that were positive for the active subunit of caspase-3 (Fig. 1a,c,c*).

3.2. z-DEVD-fmk prevents apoptosis induced by SE

The extent of apoptosis after SE was examined by electrophoretic analysis of internucleosomal DNA fragmentation and by immunohistochemical labeling of neu-

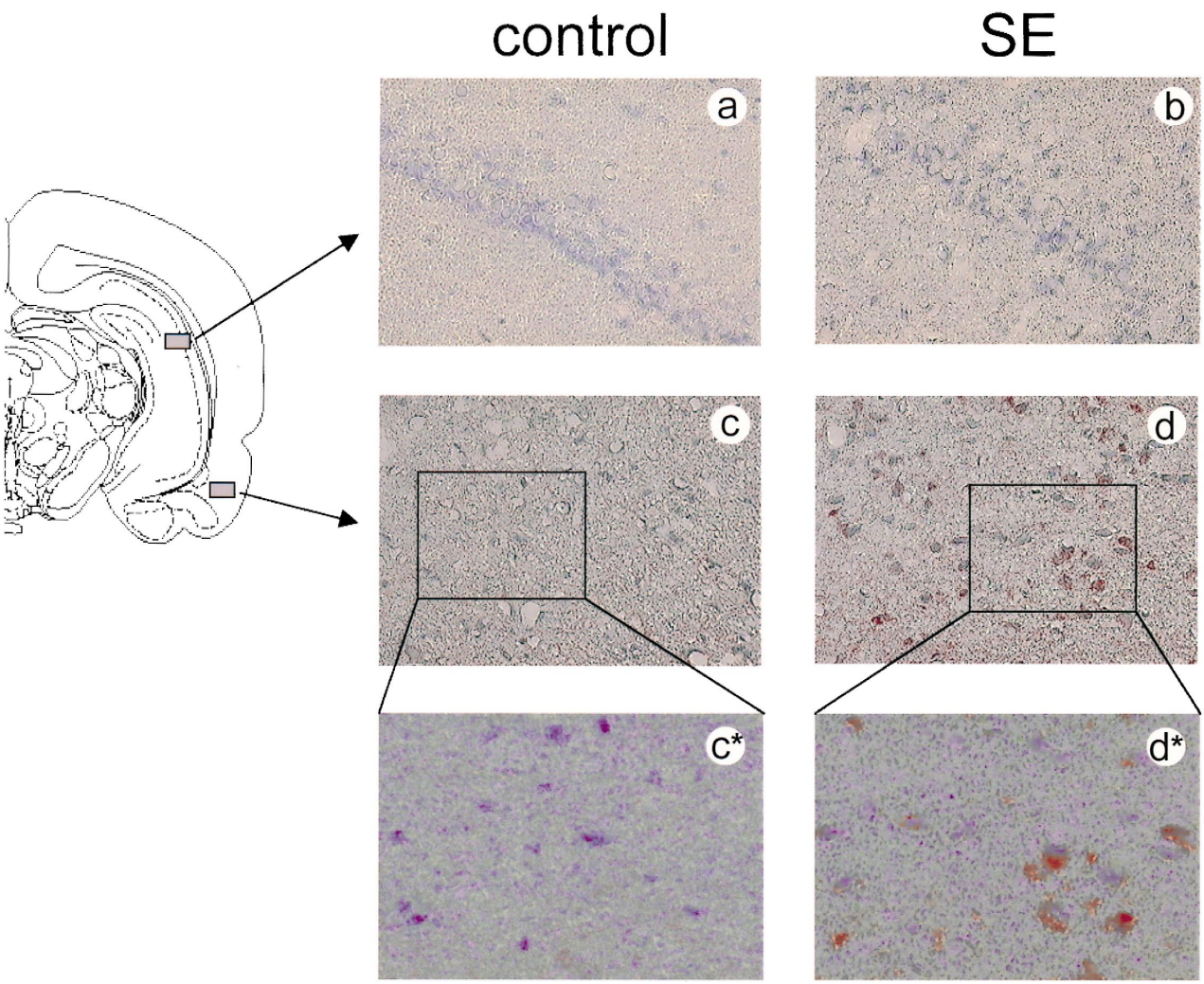

Fig. 1. Activation of caspase-3 24 h after a 2-h period of SE, as compared to saline-treated control animals not experiencing seizures. Sections from hippocampus (a, b) and entorhinal cortex (c, d) were stained with CM1 anti-p17 as described in Section 2. Cells positive for active caspase-3 (d, d*) seen as brown-red on gray-blue background. Photographs a, b, c, and d were taken at 200 × magnification. Insets c* and d* are photomicrographs of entorhinal cortex taken at 400 × magnification.

ronal nuclear morphology. Robust DNA fragmentation was detected by agarose gel electrophoresis from rhinal cortex and hippocampus taken at 24 h after a 2-h period of SE (Fig. 2A, lanes 3 and 4). Control brains (sham-operated, vehicle and diazepam-treated, without SE) exhibited barely detectable DNA fragmentation (Fig. 2A, lanes 1 and 2). In all groups of rats, the optical density of radiolabeled DNA bands was equivalent across the two hemispheres (Fig. 2B), indicating that the extent of DNA fragmentation is bilaterally symmetrical.

For immunohistochemical labeling, we used Hoechst 33258 to resolve nuclear morphology and to identify cells undergoing apoptosis. Double immunofluorescence staining with antibodies to neuron-specific nuclear protein NeuN performed on tissue taken 24-h after a 2-h period of SE confirmed that the majority of cells with apoptosis-like nuclear morphology were neurons of CA pyramidal cell layers of the hippocampus (Fig. 3A), as well as cells throughout rhinal cortex (not shown). No apoptotic-like cells were found in the brain sections from sham-operated, vehicle-treated animals not experiencing SE (not shown).

In animals treated with z-DEVD-fmk, in the hemisphere ipsilateral to the intracerebroventricular infusion of z-DEVD-fmk (see Section 2 for injection schedule), the neuronal apoptosis associated with SE was markedly attenuated (Fig. 3B); DNA fragmentation was not significantly

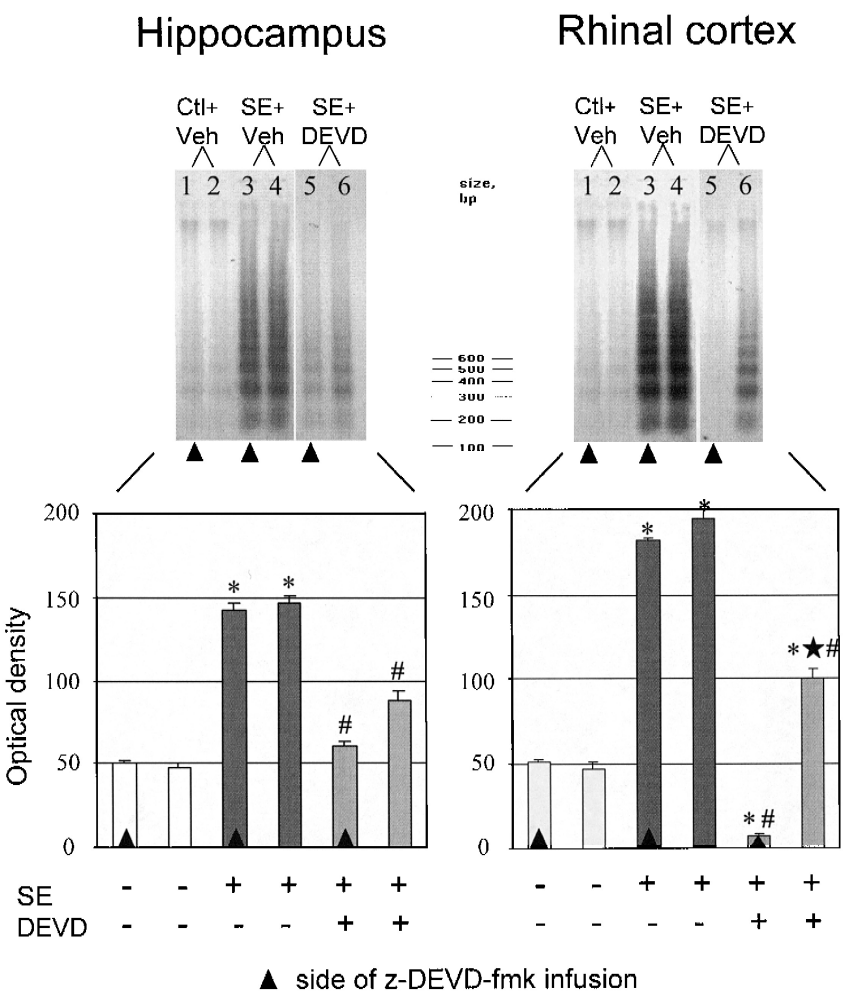

Fig. 2. DNA fragmentation evaluated 24 h after a 2-h period of SE in rats with and without z-DEVD-fmk (DEVD) treatment (see Section 2 for treatment regimen). Genomic DNA was analyzed by agarose gel electrophoresis of tissue extracts obtained from hippocampus and rhinal cortex taken from seizure-naive control animals 6 h after the last intraventricular vehicle (DMSO) treatment (see Section 2), (Ctl + Veh, lanes 1, 2), 24 h after termination of SE in rats treated with intraventricular DMSO (SE + Veh, lanes 3, 4), and 24 h after termination of SE in rats treated with intraventricular z-DEVD-fmk (SE + DEVD, lanes 5, 6). Odd numbered lanes represent the hemisphere ipsilateral to treatment infusion. Even numbered lanes are from the contralateral side. Gel photographs are representative of the other gels run for the same experiment. Note the residual DNA laddering present in hippocampal samples and in sample from rhinal cortex contralateral to the injection side taken from rats that were treated with z-DEVD-fmk. Charts below photographs represent changes in optical density (OD) of nucleosomal dimer after SE (solid bars) and after SE in the presence of z-DEVD-fmk (gray bars), as compared to vehicle-treated controls (white bars). Significant difference ($p < 0.05$) between vehicle-treated controls and SE + vehicle or SE + z-DEVD-fmk treated animals is marked by ''*'', between SE treated and z-DEVD-fmk treated animals marked by ''#'', and between hemispheres within the same treatment group, marked by ''★''.

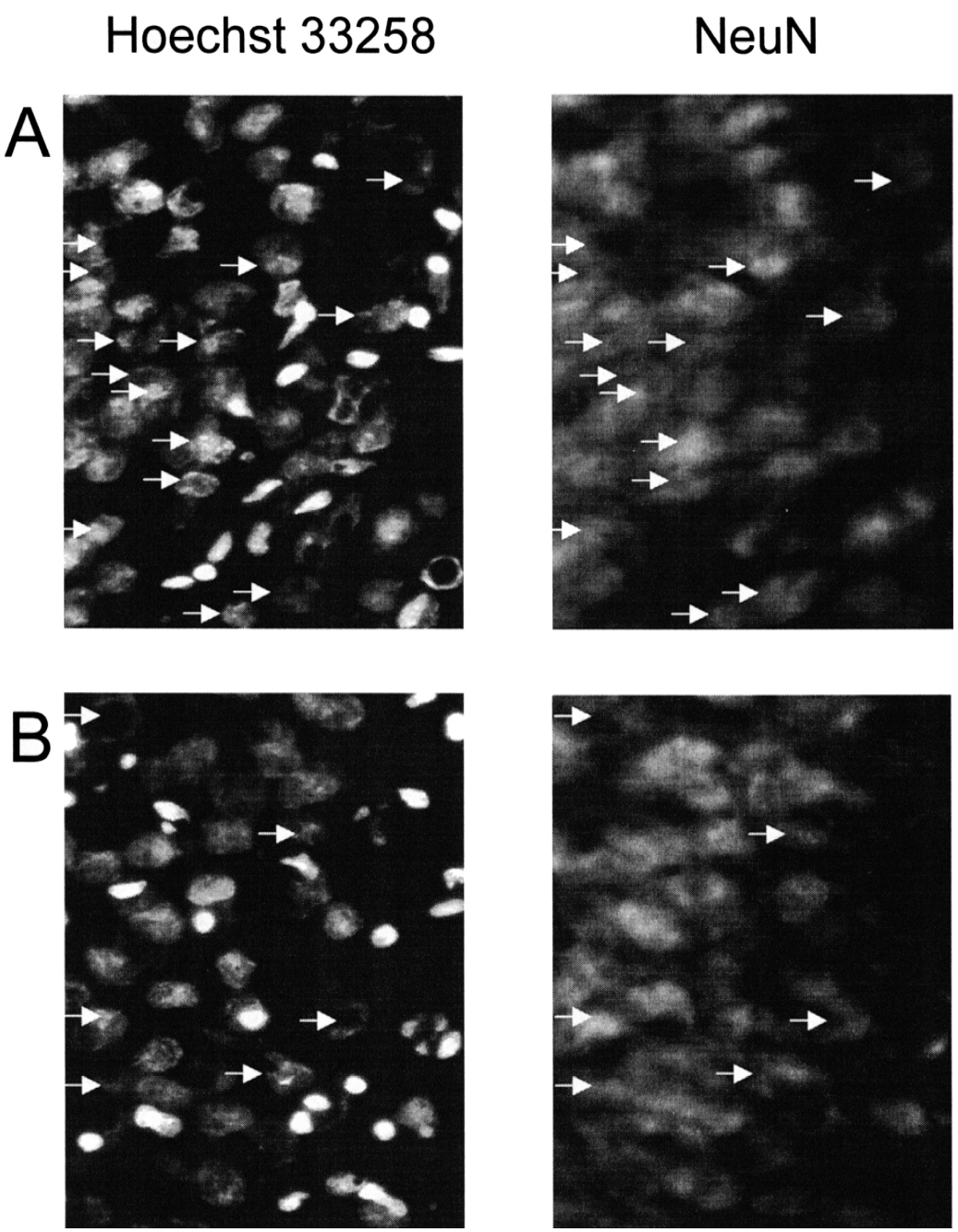

Fig. 3. Effect of z-DEVD-fmk on neuronal apoptosis in CA2 pyramidal cells. SE was induced by administration of kainic acid in (A) vehicle-treated (intraventricular DMSO) rats or (B) rats treated with intraventricular z-DEVD-fmk. Cells with apoptosis-like morphology are indicated by arrows. As evidenced by the Hoechst 33258 staining, sections obtained from control (saline-treated, seizure-naive) animals exhibited no apoptotic morphology (not shown). Many cells exhibited apoptosis-like morphology after SE as demonstrated by nuclear staining with Hoechst 33258 (left panels). The same microscopic fields were stained for antineuronal nuclear protein (NeuN) (right panels). Double immunofluorescence revealed that a majority of apoptotic calls were neurons. Photographs were taken at 400 × magnification. Note the presence of apoptotic like neurons still present after SE in the presence of z-DEVD-fmk. These cells may be responsible for residual DNA fragmentation seen on Fig. 2 (lanes 5, 6).

above the control level in the hippocampus, and completely undetectable in rhinal cortex (Fig. 2A, lanes 5). Partial protection was observed in the hemisphere contralateral to the z-DEVD-fmk infusion (Fig. 2, lanes 6), where the optical density indicative of DNA fragmentation in hippocampus and in rhinal cortex, was 39% and 51% lower, respectively, than the DNA fragmentation in the same brain regions of animals exposed to SE without z-DEVD-fmk. The extent of DNA fragmentation observed in the rhinal cortex homolateral to the z-DEVD-fmk injected ventricle was significantly less than that on the contralateral side. In contrast, there was no significant

difference between the DNA fragmentation obtained in the hippocampus of the injected hemisphere vs. contralateral hemisphere. The z-DEVD-fmk injections did not alter seizure severity, latency to seizure onset, or duration of SE following administration of kainic acid.

4. Discussion

Our results demonstrate that administration of a specific caspase-3 inhibitor, z-DEVD-fmk, significantly reduces vulnerability to the neuronal cell death that occurs in the aftermath of kainic acid-evoked SE. Furthermore, the z-DEVD-fmk exposure appears to ameliorate the apoptotic component of the neurodegenerative response, as evidenced by a significant reduction in the internucleosomal DNA fragmentation and a decreased incidence of apoptosis-like neuronal morphology in hippocampus and rhinal cortex. These data suggest that caspase-3 plays a *necessary* role in seizure-induced neurodegeneration.

The molecular and histopathological evidence presented here extends previous observations that programmed cell death (PCD) participates in the neuronal loss following experimental SE, and indicates that caspase-3-like protease plays an important role in this process. In the present study, the induction of caspase-3 activity following SE was evidenced by an increase in the amount of immunoreactive catalytic subunit (p17) in rhinal cortex and amygdala, brain areas that are especially vulnerable to SE-induced neurodegeneration. These same brain areas exhibited apoptosis-like morphological changes accompanied by internucleosomal DNA fragmentation. While we do not know the mechanism by which the cleavage of the caspase-3 precursor is initiated following SE, it is conceivable that SE-induced glutamate release could be a trigger [5]. Regions resistant to SE-evoked neurodegeneration (i.e., parietal cortex and striatum) showed no signs of p17 immunoreactivity following SE. However, two other areas that exhibit marked SE-induced apoptotic neurodegeneration (CA subfields of hippocampus and dorsomedial thalamus) (Fig. 3A and Fig. 2) also showed no signs of caspase-3 activation (Fig. 1b). Thus, whereas cell death in rhinal cortex and amygdala after SE is associated with caspase-3 activation, cell death in other areas may depend upon different members of the caspase-like preotease family.

The fact that caspase-3 is activated by prolonged seizure activity leads to the question of whether caspase-3 is a necessary component of the apoptotic response following SE. Consequently, we examined the effect of caspase-3 inhibition in vivo on the histological and biochemical manifestations of apoptosis following SE. Intracerebroventricular injection of the tetrapeptide inhibitor of caspase-3-like proteases, z-DEVD-fmk, significantly decreased the intranucleosomal DNA fragmentation and the incidence of apoptotic-like neuronal morphology following SE. The protection was observed to extend to regions (rhinal cortex) that were located a considerable distance from the intracerebroventricular injection site, indicating that the inhibition of caspase activity is effective over a several millimeter range of drug diffusion. The DMSO vehicle is likely to facilitate drug diffusion from the ventricle and throughout the brain tissue. However, while equivalent protection was observed in the hippocampus of both hemispheres, the protection observed in rhinal cortex was markedly asymmetrical, with the rhinal cortex in the hemisphere in which the infusions were made showing a significantly greater neuroprotective response (Fig. 2) than that on the contralateral side. This suggests that the concentration of z-DEVD-fmk is less than maximally inhibitory in tissues located more than 7 mm away from the intracerebroventricular injection site.

While inhibition of caspase-3 is a plausible explanation for the neuroprotective action of z-DEVD-fmk, other related proteases may also be inhibited by this agent. z-DEVD-fmk was designed to mimic the cleavage site on poly(ADP-ribose) polymerase (PARP) [22], which is a substrate for caspase-3. However, PARP is also a substrate for several caspase-3-related proteases, including caspase-6 and caspase-7 [11]. Caspase-4 and caspase-2 can also cleave PARP, albeit with very high enzyme-substrate ratios [46]. Thus, we cannot exclude the possibility that the protective effect achieved by z-DEVD-fmk is via the inhibition of a group of enzymes with caspase-3-like activity. In fact, one or more of these enzymes may play a role in cell death in hippocampus. Our failure to detect p17 immunoreactivity in hippocampus following SE suggests that caspase-3 is not the major cysteine protease in this region. Nevertheless, z-DEVD-fmk protected against cell death in the hippocampus. This apparent inconsistency may be explained by an inhibitory action of z-DEVD-fmk on enzymes with caspase-3-like activity other than caspase-3 itself. In contrast, in rhinal cortex, the elevation of p17 immunoreactivity after SE is consistent with caspase-3 inhibition accounting for the neuroprotective effect of z-DEVD-fmk in this area.

The fact that z-DEVD-fmk is also protective against neuronal death associated with other injury models, namely traumatic brain injury [43] and transient cerebral ischemia [2], indicates that the effect we have observed is not specific to the injury model. This is consistent with z-DEVD-fmk working via a common mechanism of cell death.

What is the potential mechanism by which the activation of caspase-3-like proteases may contribute to SE-evoked apoptotic cell death? It has been suggested that the activation of caspase-3, followed by cleavage of specific substrates, may contribute to the process of apoptosis by a combination of changes in signaling molecules and structural changes. One early biochemical event that accompanies apoptosis in many cell types is the caspase-3-mediated proteolytic cleavage of nuclear proteins, including PARP [25,30] and DNA-dependent protein kinase (DNA-PK) [1].

The significance of cleavage of these proteins in apoptosis currently is uncertain, although PARP and DNA-PK are involved in DNA repair and protection of fragmented DNA [27,35]. At least two important components of the cytoskeleton, β-actin and actin-associated protein α-fodrin, also are cleaved during apoptosis by caspase-3-like proteases. When cleaved by caspase-3, actin loses its ability to polymerize and to inhibit DNase I activity [24]. Thus, an activation of caspase-3-like enzyme or enzymes may contribute to both the morphological changes and the DNA fragmentation that we have observed to follow SE.

In summary, we have demonstrated that the occurrence of apoptosis in selected brain regions after SE is accompanied by the induction of caspase-3 activity. Moreover, in vivo administration of a specific tetrapeptide inhibitor of caspase-3 markedly attenuated the SE-evoked apoptosis in rhinal cortex and hippocampus. These results implicate caspase-3-like proteases as important agents in the process of neuronal apoptosis after SE, and suggest that members of the caspase-3-like protease family may regulate neuronal PCD in a regionally-specific fashion.

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