


RESEARCH ARTICLE

A role for astrocyte-derived amyloid β peptides in the degeneration of neurons in an animal model of temporal lobe epilepsy

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Keywords

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Abstract

Kainic acid, an analogue of the excitatory neurotransmitter glutamate, can trigger seizures and neurotoxicity in the hippocampus and other limbic structures in a manner that mirrors the neuropathology of human temporal lobe epilepsy (TLE). However, the underlying mechanisms associated with the neurotoxicity remain unclear. Since amyloid- β ($A\beta$) peptides, which are critical in the development of Alzheimer's disease, can mediate toxicity by activating glutamatergic NMDA receptors, it is likely that the enhanced glutamatergic transmission that renders hippocampal neurons vulnerable to kainic acid treatment may involve $A\beta$ peptides. Thus, we seek to establish what role $A\beta$ plays in kainic acid-induced toxicity using *in vivo* and *in vitro* paradigms. Our results show that systemic injection of kainic acid to adult rats triggers seizures, gliosis and loss of hippocampal neurons, along with increased levels/processing of amyloid precursor protein (APP), resulting in the enhanced production of $A\beta$ -related peptides. The changes in APP levels/processing were evident primarily in activated astrocytes, implying a role for astrocytic $A\beta$ in kainic acid-induced toxicity. Accordingly, we showed that treating rat primary cultured astrocytes with kainic acid can lead to increased $A\beta$ production/secretion without any compromise in cell viability. Additionally, we revealed that kainic acid reduces neuronal viability more in neuronal/astrocyte co-cultures than in pure neuronal culture, and this is attenuated by precluding $A\beta$ production. Collectively, these results indicate that increased production/secretion of $A\beta$ -related peptides from activated astrocytes can contribute to neurotoxicity in kainic acid-treated rats. Since kainic acid administration can lead to neuropathological changes resembling TLE, it is likely that APP/ $A\beta$ peptides derived from astrocytes may have a role in TLE pathogenesis.

Abbreviations

$A\beta$ = amyloid β ; AD = Alzheimer's disease; APH1 = anterior pharynx defective 1; APP = amyloid precursor protein; BACE1 = β -site APP cleaving enzyme; BCA = bicinchoninic acid; CTF = C-terminal fragment; ECL = enhanced chemiluminescence; ERK1/2 = extracellular signal-regulated kinases 1/2; GFAP = glial fibrillary acidic protein; HRP = horseradish peroxidase; MAP kinase = mitogen activated protein kinase; MTT = 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide; PBS = phosphate-buffered saline; PEN2 = presenilin enhancer 2; PFA = paraformaldehyde; PS1/2 = presenilins 1/2; TLE = temporal lobe epilepsy; SBDP = α II-spectrin breakdown products

INTRODUCTION

Kainic acid, an analogue of the excitatory neurotransmitter glutamate, can induce seizures in adult rats originating from the CA3 region of the hippocampus and then spreading to other limbic structures. The epileptogenic effects of kainic acid are predominantly caused by the activation of ionotropic kainate receptors located on CA3 mossy fiber synapses. This triggers Ca^{+2} influxes into the cells, generating synchronized, network-driven glutamatergic currents which

subsequently induce seizures and facilitate neurotoxicity (10, 44, 80). The consequences of kainic acid administration include neuronal loss in CA1-CA3 regions, the reorganization of mossy fibers and gliosis; neuropathologies which resemble human temporal lobe epilepsy (TLE) (45, 89). Glutamate, derived from both synaptic and non-synaptic (ie, astrocytes) sources, plays a critical role in kainic acid-induced epilepsy, as its administration can induce seizures and loss of neurons in animals, which can be precluded by glutamate receptor

Table 1. Primary antibodies used.

Antibody	Type	IHC/IF dilution	WB dilution	Source
α -Fodrin	Monoclonal	n/a	1:1000	Enzo Life Sciences, Inc.
β -actin	Monoclonal	n/a	1:5000	Sigma-Aldrich, Inc.
μ -Calpain	Polyclonal	n/a	1:1000	Sigma-Aldrich, Inc.
APH1	Polyclonal	1:200	1:1000	Kind gift from Dr. G. Thinakaran, The University of Chicago, US
APP (clone Y188)	Monoclonal	n/a	1:5000	Abcam Inc.
APP (clone 369)	Polyclonal	n/a	1:1000	Kind gift from Dr. G. Thinakaran, The University of Chicago, US
APP (clone CTM1)	Polyclonal	1:2000	n/a	Kind gift from Dr. G. Thinakaran, The University of Chicago, US
BACE1	Polyclonal	1:200	1:1000	EMD Millipore Co.
CD68 (clone ED1)	Monoclonal	1:50	n/a	AbD Serotec
Cleaved Caspase-3	Monoclonal	1:200	n/a	Cell Signaling Technology
GFAP	Polyclonal and monoclonal	1:500	n/a	Dako
Nicastrin (N-19)	Polyclonal	1:200	1:500	Santa Cruz Biotechnology, Inc.
p44/42 MAPK (ERK1/2)	Monoclonal	n/a	1:1000	Cell Signaling Technology, Inc.
Phospho-p44/42 MAPK (ERK1/2)	Polyclonal	n/a	1:1000	Cell Signaling Technology, Inc.
Phospho-CREB (Ser133)	Monoclonal	n/a	1:1000	Cell Signaling Technology, Inc.
PEN2	Polyclonal	1:200	1:1000	Kind gift from Dr. G. Thinakaran, The University of Chicago, US
PS1	Polyclonal	1:500	1:3000	Kind gift from Dr. G. Thinakaran, The University of Chicago, US
sAPP α (2B3)	Monoclonal	n/a	1:1000	IBL Co., Ltd
sAPP β (Wild-type)	Monoclonal	n/a	1:1000	IBL Co., Ltd

Abbreviations: IHC/IF = Immunohistochemistry/Immunofluorescence; WB = western blotting; n/a = not used in specified application.

antagonists (19, 22, 29). Nevertheless, the cellular mechanisms by which kainic acid triggers cell death remain unclear as apoptotic, necrotic and autophagic markers are found to be altered in affected neurons (81, 82). Thus, defining the signaling pathways that underlie neurotoxicity following kainic acid-induced seizure may provide a better understanding of the pathology associated with TLE.

Numerous studies have shown that amyloid β (A β) peptides, produced constitutively in the normal brain, play a critical role in reducing neuronal viability and subsequent development of Alzheimer's disease (AD), the most common type of senile dementia affecting the elderly (21, 48). These peptides are derived from the amyloid precursor protein (APP), which can be proteolytically processed either by non-amyloidogenic α -secretase or amyloidogenic β -secretase pathways. The α -secretase (eg, ADAM10) cleaves APP within the A β domain, yielding soluble N-terminal APP α (sAPP α) and a C-terminal fragment (α -CTF) that can be further processed by γ -secretase to generate A β_{17-40} /A β_{17-42} fragments. The β -secretase cleaves APP to generate soluble APP β (sAPP β) and an A β -containing C-terminal fragment (β -CTF), which is processed via γ -secretase to yield full-length A β_{1-40} /A β_{1-42} peptides. While β -secretase is an aspartyl protease called β -site APP cleaving enzyme (BACE1), γ -secretase comprises the aspartyl protease presenilin 1/2 (PS1/PS2) and three cofactors, that is, nicastrin, presenilin enhancer 2 (PEN2) and anterior pharynx defective 1 (APH1) (3, 28).

There is now considerable evidence suggesting that toxicity induced by A β -related peptides is partly mediated by glutamate, as transient inactivation and/or blockade of the NMDA receptor by antagonists can protect neurons against A β toxicity (1, 40, 75, 78, 85). These results raise the possibility that enhanced glutamatergic activity that renders hippocampal neurons vulnerable to excitotoxicity in animal models of TLE (50, 67, 82) may involve A β peptides. This is supported by three lines of evidence (i) prolonged activation of glutamatergic NMDA receptors can enhance amyloidogenic processing of APP (13, 43, 64), (ii) neuronal vulnerability to excitotoxicity is

enhanced in the presence of A β (53) and (iii) transgenic mice overexpressing A β exhibit spontaneous seizures or decreased threshold to seizures compared to wild-type mice (20, 58, 87). Nevertheless, little is known regarding the involvement of A β peptides in the loss of neurons following kainic acid-induced toxicity. To address this issue, we evaluated alterations in the levels/distribution and processing of APP in the hippocampus of kainic acid-treated rats. In parallel, using rat hippocampal neuronal and mixed hippocampal neuronal/astrocyte cultures, we determined the significance of A β peptides in kainic acid-mediated neurotoxicity. Collectively, our results reveal that increased levels of astrocyte-derived A β peptides have a role in reducing neuronal viability in the kainic acid model of TLE.

MATERIALS AND METHODS

Materials

Polyacrylamide electrophoresis gels (4%–20%), cell culture reagents such as Hank's balanced salt solution, fetal bovine serum, Neurobasal media, B27 and N2 supplements, and ELISA kits for the detection of rat A β_{1-40} and A β_{1-42} were purchased from Invitrogen (Burlington, ON, Canada), whereas the enhanced chemiluminescence kit (ECL) and the bicinchoninic acid (BCA) protein assay kit were from Thermo Fisher Scientific (Montreal, QC, Canada). Kainic acid, γ -secretase inhibitors (DAPT and L-685,458) and 3-(4,5-dimethylthiozoly)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich (Oakville, ON, Canada). Human and rat A β_{1-42} and the reverse sequence A β_{42-1} were purchased from American Peptides (Sunnyvale, CA, USA) and the Live/Dead cell viability assay kit was from Molecular Probes (Eugene, OR, USA). Fluoro-Jade C was from Chemicon Int., (Temecula, CA, USA), whereas β -secretase BACE1 activity assay kit was from Abcam (Cambridge, United Kingdom). Sources of all primary antibodies used in the study are listed in Table 1. All

horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology (Paso Robles, CA, USA). Vivaspin filtration columns were from GE Healthcare Ltd. (Mississauga, ON, Canada), whereas protease inhibitor cocktail, BACE1 inhibitor BIV and fluorogenic γ -secretase substrate were from Calbiochem (San Diego, CA, USA). All other chemicals were from either Sigma-Aldrich or Thermo Fisher Scientific.

Systemic administration of kainic acid

Adult male Sprague-Dawley rats obtained from Charles River (St Constant, QC, Canada) were housed with access to food and water *ad libitum*. The experiments were performed in accordance with Institutional and Canadian Council on Animal Care guidelines. Adult rats were injected intraperitoneally with either kainic acid dissolved in normal saline (12 mg/kg) or equal volume (0.2–0.25 mL) normal saline as described previously (8, 79). Following treatment, the control and kainic acid-treated rats were euthanized at 12 h, 2, and 12 days by decapitation; their hippocampi were dissected out of the brain and frozen immediately in dry-ice for biochemical assays. For histological studies, control and kainic acid-treated rats were anesthetized with 8% chloral hydrate and then perfused with phosphate-buffered saline (PBS), followed by 4% paraformaldehyde (PFA). Brains were sectioned (20 or 40 μ m) on a cryostat and collected in a free-floating manner for further processing.

Fluoro-Jade C Staining

Fluoro-Jade C labels degenerating neurons, dendrites and axons (68). Sections from control and kainic acid-treated rat brains from various time periods were stained with 0.0001% Fluoro-Jade C in 0.1% acetic acid as described earlier (38).

Immunostaining

Brain sections from control and kainic acid-treated rats (3–5 animals/group) were processed as described earlier (38). For the enzyme-linked procedure, 40 μ m sections were incubated overnight at 4°C with anti-APP (CTM1), anti-BACE1, anti-PS1, anti-nicastrin, anti-PEN2 or anti-APH1 antiserum at dilutions listed in Table 1. Sections were then exposed to HRP-conjugated secondary antibodies for 1 h and developed using the glucose-oxidase-nickel enhancement method. For double immunofluorescence staining, brain sections (20 μ m) from control and kainic acid-treated rats were sequentially labeled with anti-APP (CTM1), anti-BACE1, anti-PS1, anti-nicastrin, anti-PEN2 or anti-APH1 antisera, followed by anti-ED1, anti-glial fibrillary acidic protein (GFAP) or anti-cleaved caspase-3 antisera at dilutions listed in Table 1. The primary antibodies were labeled with either Texas Red- or FITC-conjugated secondary antibodies (1:200), washed and then mounted with Vectashield medium. Immunostained sections were examined and photographed using a Zeiss Axioskop-2 fluorescence microscope (49).

Immunoblotting

Hippocampal tissues from control and kainic acid-treated rats from different time periods (6–8 animals/group) were homogenized in ice-cold radioimmunoprecipitation assay-lysis buffer and protein

content was determined using a BCA kit. The proteins were separated by electrophoresis on 4%–20% polyacrylamide gels and then transferred to nitrocellulose membranes. The membranes were blocked with 5% non-fat milk and incubated overnight at 4°C with anti-APP (369), anti-BACE1, anti-PS1, anti-nicastrin, anti-PEN2, anti-APH1, anti-sAPP α , anti-sAPP β , anti-Y1888, anti-extracellular signal-regulated kinases 1/2 (ERK1/2), anti-phospho-cAMP response element-binding protein (phospho-CREB), anti- α -fodrin, anti- μ -calpain and anti-cleaved caspase-3 antibodies at dilutions listed in Table 1. Membranes were then incubated with the HRP-conjugated secondary antibodies and developed using an ECL detection kit. Membranes were subsequently reprobed with either anti- β -actin or anti-total ERK1/2 antibody and immunoreactive proteins were quantified using MCID image analysis system (37).

β - and γ -secretase activity assays

The β -secretase activity in the hippocampus of control and kainic acid-treated rats (4–6 animals/group) was measured using the fluorometric β -secretase activity assay kit according to the manufacturer's instructions as described earlier (83). The fluorescence was measured at excitation wavelength of 355 nm and emission wavelength of 495 nm and specific activity of the enzyme was determined by incubating parallel samples with a β -secretase inhibitor BIV. The γ -secretase activity assay was performed (4–6 animals/group) as described previously (49). The fluorescence was measured at an excitation wavelength of 355 nm and emission wavelength of 440 nm at 37°C and the specific activity was determined by incubating a parallel set of samples with 100 μ M γ -secretase inhibitor L-658,458. All samples were assayed in duplicate and data were obtained from three independent experiments.

Rat A β _{1–40} and A β _{1–42} levels

A β peptides from hippocampus of control and kainic acid-treated rats (4–6 animals/group) were solubilized in 5M guanidine buffer (16), centrifuged and then assayed for rat A β _{1–40} or A β _{1–42} levels using ELISA kits as described earlier (49). In a separate series of experiments, cellular and secretory levels of A β _{1–40} and A β _{1–42} were measured in cultured astrocytes treated with or without kainic acid in a similar manner. All samples were assayed in duplicate and the data were obtained from three independent experiments.

Primary hippocampal neuronal, astrocyte and astrocyte/neuronal cultures

Primary rat hippocampal neuronal cultures were prepared from 17/18-day-old embryos of time-pregnant Sprague-Dawley rats as described previously (86, 96). In brief, the hippocampal region was dissected in Hanks balanced salt solution supplemented with 100 μ M HEPES, 10 mM Na-pyruvate, 50 μ M L-glutamine, 10 U/mL penicillin and 10 mg/mL streptomycin and digested with 0.25% trypsin/EDTA. The cell suspension was filtered through a cell strainer and then plated on either 96-well plate or 12 mm glass coverslips. The cultures were grown at 37°C in a 5% CO₂-humidified atmosphere in Neurobasal medium supplemented with B27, 50 μ M L-glutamine, 100 μ M HEPES, 10 mM Na-pyruvate, 10 U/mL penicillin and 10 mg/mL streptomycin, and 1% FBS. The medium was replaced 1 day later without FBS and all experiments were performed on day 6 after plating. Primary rat hippocampal astrocytes

(Sciencell, CA, USA) were seeded on Poly-D-lysine-coated plates and grown in the associated astrocyte media at 37°C as per the manufacturer's instructions. Cells were grown to confluency and passaged using TrypLE Express. To prepare astrocyte-neuronal co-cultures, hippocampal neurons were prepared as described above, and then seeded directly on a layer of astrocytes grown to confluency. Media conditions for co-cultures follow the protocol for neuronal culture and all experiments were performed 6 days following cell plating.

Treatment of cultured neurons, astrocytes and mixed neurons/astrocytes

The oligomeric forms of rat and human A β_{1-42} used in all experiments related to toxicity were prepared as described earlier (75) and added to neurons to the final concentrations (0.01–20 μ M) for different periods of time (ie, 12, 24, 48 or 72 h). Control and A β -treated cultures were then processed for MTT cell viability and live/dead cell toxicity assays (75). In a separate set of experiments, cultured rat hippocampal astrocytes were treated with or without 20 μ M γ -secretase inhibitor DAPT for 24 h and then exposed to 100 μ M kainic acid for an additional 24 h. Subsequently, the cellular and secretory levels of rat A β_{1-40} /A β_{1-42} as well as cell viability were measured. In parallel, cultured neurons and mixed neurons/astrocytes were exposed to 100 μ M kainic acid with or without 20 μ M DAPT for 24 or 48 h and their cell viability was assessed (49).

Cell viability assays

Viability of neurons or astrocytes was assessed using the cell proliferation colorimetric MTT assay (83). Control and treated culture plates were replaced with new medium containing 0.25% MTT and then incubated for 2 h at 37°C. The reaction was terminated and measured spectrophotometrically at 570 nm. Neuronal viability following treatment with A β peptide was also assessed using the Live/Dead assay kit containing calcein AM and ethidium homodimer (EthD-1) as the fluorescent probes. Control and treated cultured neurons were incubated with medium containing 2 μ M calcein AM and 4 μ M EthD-1 for 30 min at 37°C, fixed in 4% PFA and then visualized under a Zeiss Axioskop-2 fluorescent microscope (75).

Statistical analysis

Data are expressed as means \pm SEM. Statistical significance of differences was determined by one-way ANOVA followed by Newman-Keuls *post hoc* analysis for multiple comparisons or unpaired two-tailed Student's *t* test for single comparison with a significance threshold of $P < 0.05$. All analyses were performed using GraphPad Prism Software.

RESULTS

Loss of neurons and proliferation of glial cells in kainic acid-treated rats

In keeping with earlier studies (8, 10, 45, 80), we observed that systemic administration of kainic acid evoked seizures characterized by rearing and falling, facial and forelimb clonus, and “wet-dog” shakes that lasted about 8–10 h. After a seizure-free period lasting

a few days, a high proportion of animals surviving such an attack exhibited a chronic phase of spontaneous recurrent limbic seizures with no remission over 12 days of the experimental paradigm (18, 89). These behavioral changes are accompanied by a loss of neurons, as apparent by Fluoro-Jade C and cleaved caspase-3-labeling, as well as glial hypertrophy in the hippocampus of the rat brain (Figure 1A–K). As expected, reduced neuronal viability progressed with time primarily in CA1–CA3 pyramidal cells and the hilar region of the dentate gyrus, whereas the granule cell layer was relatively spared (8, 80, 84). The hypertrophy of astrocytes and microglia (data not shown) was apparent to some extent at 12 h and then gradually increased over 12 days post-treatment period (19, 35, 80) (Figure 1A–I). The progressive loss of neurons and activation of glial cells were seen in the hippocampi of both hemispheres in kainic acid-treated rats.

Earlier studies have indicated that kainic acid induces neurotoxicity by increasing Ca²⁺ influx, the production of reactive oxygen species and the activation of multiple downstream signaling cascades including mitogen activated protein (MAP) kinase and the cysteine protease calpain (4, 10, 11, 34, 80, 82, 93). While calpain exists in two different isoforms (μ - and m-calpains), the family of MAP kinase is composed of various enzymes such as stress-activated protein kinases and ERK1/2 (34, 80, 93). A significant induction of phospho-ERK1/2 and its downstream substrate phospho-CREB was evident following kainic acid administration at 12 h and 2 days, respectively, and remained elevated over the 12 day post-treatment period (Figure 2A). Concurrently, we also observed a protracted increase in calpain levels in the hippocampus of kainic acid-treated rats. To validate the activation of calpain, we measured the breakdown products of non-erythroid α II-spectrin (ie, fodrin)—a signature downstream substrate which yields unique proteolytic fragments following cleavage by caspase and calpain (4, 11, 82). While calpain-specific 150/145kD α II-spectrin breakdown products (SBDP) were increased at all time points following administration of kainic acid, with maximal changes apparent at 2 day post-treatment, caspase-3 specific 120 kD SBDP showed alterations mostly at 2 day post-treatment over the 12 days experimental paradigm (Figure 2B). Caspase activation is further supported by our Western blot (Figure 2B) and the detection of immunoreactive cleaved-caspase-3-positive neurons (Figure 1J,K) in kainic acid-treated rat hippocampus compared to control rats.

Increased levels and expression of APP and its processing enzymes in kainic acid-treated rats

APP levels were markedly increased at 2 and 12 days, but not at 12 h, following kainic acid administration compared to control rats (Figure 3A). At the cellular level, APP immunoreactivity in the control hippocampus was evident mostly in CA1–CA3 pyramidal neurons and granule cells of the dentate gyrus but not in glial cells (Figure 4A,B; Supporting Information Figure S1A–D). Occasionally, APP-immunoreactive neurons were also apparent in the hilus region of the dentate gyrus (Figure 4A). While the overall expression of APP decreased with time in the degenerating hippocampal neurons, a number of glial cells were found to express APP from 2 days onwards after kainic acid treatment (Figure 4C–F). Our double labeling showed that immunoreactive APP is evident in a subset of GFAP-labeled

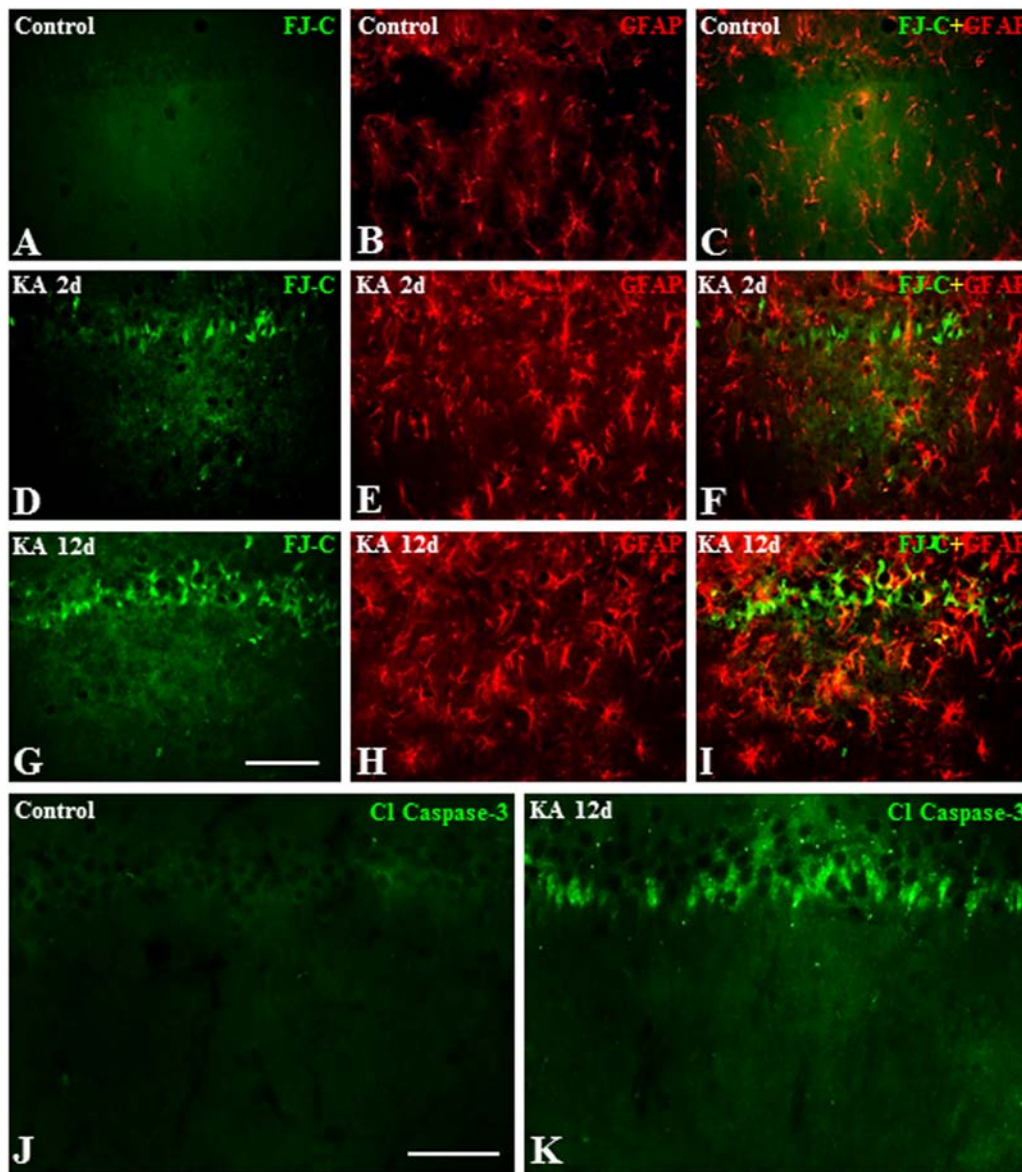


Figure 1. A–I. Photomicrographs depicting Fluoro-Jade C labeled neurons (A, D, G), GFAP-labeled astrocytes (B, E, H) and their spatial co-distribution (C, F, I) in the hippocampus of control (A–C), 2day (D–F) and 12day (G–I) kainic acid-treated rats. Note the lack of Fluoro-Jade C labeling and proliferation of astrocytes in control rat hippocampus (A–C), whereas the loss of neurons and proliferation of astrocytes

increased gradually in the hippocampus of kainic acid-treated rats over 12day post-treatment period. J and K, Photomicrographs depicting cleaved-caspase-3 labeling in the hippocampus of control (J) and 12day kainic acid-treated (K) rats. Note the cleaved-caspase-3 labeled neurons following administration of kainic acid but not in control rat. Scale bar, 100 μ M.

reactive astrocytes, but not ED1-labeled activated microglia, following kainic acid treatment (Figure 4G–J).

To determine if enhanced APP levels in kainic acid-treated rats were accompanied by a parallel increase in APP processing via the β -secretase pathway, we measured the levels/expression of BACE1 and four components of the γ -secretase complex (Figures 3B, Figures 5–6; Supporting Information Figures S1–S5) in the hippocampus of treated and control rats. We observed a significant increase in BACE1 levels at 2 and 12 days following kainic acid treatment compared to control rats (Figure 3B).

The components of the γ -secretase complex, PS1 (Figure 3C), nicastrin (Figure 3D), PEN2 (Figure 3E) and APH1 (Figure 3F) were likewise found to be enhanced in kainic acid-treated rats. Consistent with earlier studies (37, 71), immunoreactive BACE1 and four components of γ -secretase complex were evident mostly in neurons of the normal brain. In the hippocampal region, intense immunoreactivity for BACE1 (Figure. 5A,B; Supporting Information Figure S1E–H) and components of γ -secretase complex, that is, PS1 (Figure 6A,B; Supporting Information Figure S1I–L), nicastrin (Supporting Information

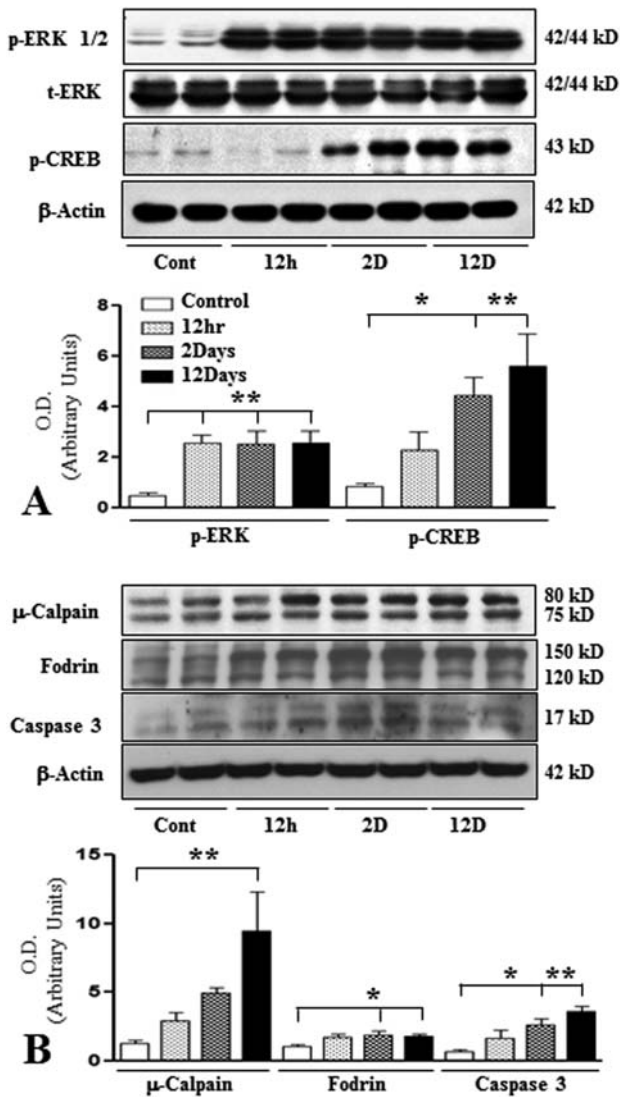


Figure 2. **A.** Histogram and representative immunoblots showing the levels of phospho-ERK1/2, total ERK, phospho-CREB in the hippocampus of control and kainic acid-treated rats. Note the relative increase in the levels of these markers following treatment with kainic acid compared to saline-treated control rats. **B.** Histogram and representative immunoblots showing the levels of μ -calpain, fodrin, caspase 3 in the hippocampus of control and kainic acid-treated rats. Note the relative increase in the levels of these markers following treatment with kainic acid compared to saline-treated control rats. All blots were re-probed with anti- β -actin to monitor protein loading. Values represent means \pm SEM from 6–8 rats. * P <0.05, ** P <0.01.

Figures S2A,B and S5A–D), PEN2 (Supporting Information Figures S3A,B and S5E–H) and APH1 (Supporting Information Figures 4A,B and S5I–L) was apparent in the CA1-CA3 pyramidal cell layer and in a few medium-sized neurons scattered in the strata oriens and stratum radiatum but not in glial cells. Within the dentate gyrus, granule cell somata were outlined by a fine mesh of weakly stained puncta and occasional strongly labeled neurons. In comparison with the control hippocampus, a number of glial cells were found to express immunoreactive

BACE1 (Figure 5C–F) and components of γ -secretase complex in kainic acid-treated rats from 2 day post-treatment onwards (Figure 6C–F; Supporting Information Fig. S2C–F, S3C–F and S4C–F). Our double immunolabeling revealed that a subset of reactive astrocytes express immunoreactive BACE1 (Figure 5G,H), PS1 (Fig. 6G, H), nicastrin (Supporting Information Figure S2G,H), PEN2 (Supporting Information Figure S3G,H) and APH1 (Supporting Information Figure S4G,H) in the hippocampus of treated rats. Activated microglia, conversely, did not express either BACE1 (Figure 5I,J) or components of γ -secretase, that is, PS1 (Figure 6I,J), nicastrin (Supporting Information Figure S2I,J), PEN2 (Supporting Information Figure S3I,J) and APH1 (Supporting Information Figure S4I,J) at any time points following kainic acid administration.

APP processing and enhanced rat A β_{1-40} /A β_{1-42} levels in kainic acid-treated rats

To determine whether kainic acid treatment can influence APP processing, we first measured the activity of β - and γ -secretases, as well as the steady state levels of APP-cleaved products (sAPP α , sAPP β , α -CTF and β -CTF) in the hippocampus of treated and control rats. Our results showed that BACE1 and γ -secretase activities were significantly increased in time-dependent manner following kainic acid administration (Figure 7A,B). Accompanying these data, levels of sAPP β were increased from 2 day post-treatment, while sAPP α levels were not altered at any time relative to the control rats (Figure 7C). The levels of α -CTF and β -CTF were also significantly increased after kainic acid treatment, but the changes observed for β -CTF were found to be somewhat higher than α -CTF (Figure 7D). In parallel, rat A β_{1-40} and A β_{1-42} levels, as detected by ELISA, were markedly increased from 2 day onwards in the hippocampus of kainic acid-treated rats compared to control rats (Figure. 7E,F).

Rat A β_{1-42} is toxic to primary rat hippocampal neurons

Consistent with earlier results (39, 75), chronic exposure to oligomeric human A β_{1-42} reduced viability of hippocampal neurons in a concentration-dependent manner, as evident by a reduction in MTT values (Supporting Information Figure S6A). To determine whether rat A β_{1-42} , which differs from the human A β_{1-42} by three amino acids (15), is toxic to cells, we first evaluated its dose-dependent (1–20 μ M) effect on hippocampal neurons. The viability of neurons, as indicated by MTT assay, was not affected by rat 1–10 μ M A β_{1-42} , but significantly decreased with 20 μ M A β_{1-42} following 24 h treatment (Supporting Information Figure S6B). The toxic potency of rat A β_{1-42} was further substantiated by a time-dependent decrease in MTT value, with ~ 50% cell death observed at 72 h post-treatment (Supporting Information Figure S6C). Our live-dead assay also showed an increase in the number of dead cells following exposure to 20 μ M rat A β_{1-42} for 24 h (Supporting Information Figure S6D–G).

Kainic acid treatment enhanced A β_{1-40} /A β_{1-42} levels in cultured rat astrocytes

Our *in vivo* studies indicated that kainic acid-induced neurotoxicity was accompanied by increased levels/processing of APP,

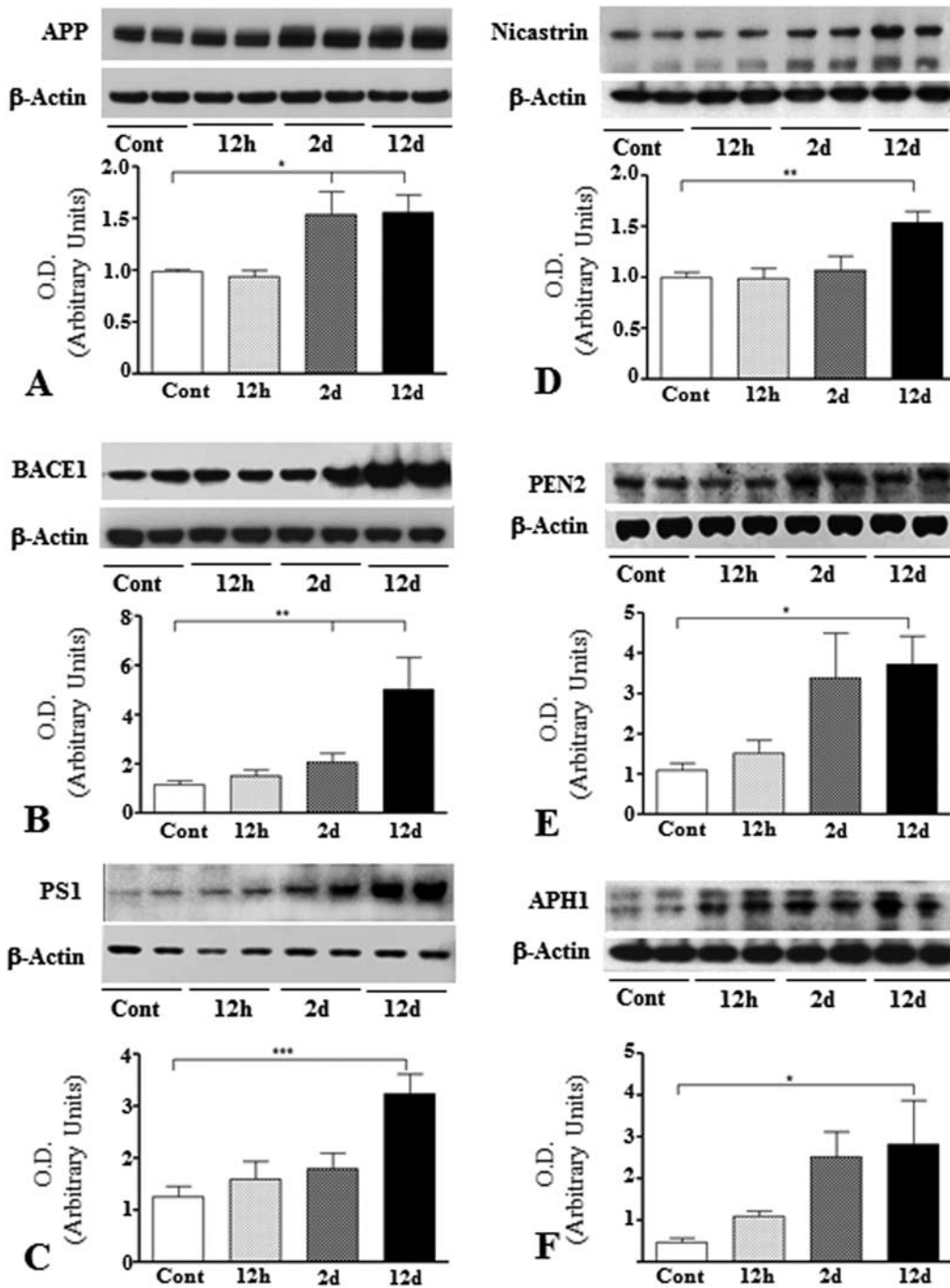


Figure 3. Histograms and representative immunoblots showing the levels of APP (A), BACE1 (B), PS1 (C), nicastrin (D), PEN2 (E) and APH1 (F) in the hippocampus of control and kainic acid-treated rats. Note the time-dependent increase in the levels of APP, BACE1 and

four components of the γ -secretase complex following treatment with kainic acid compared to saline-treated control rats. All blots were reprobed with anti- β -actin to monitor protein loading. Values represent means \pm SEM from 6–8 rats. * P <0.05, ** P <0.01, *** P <0.001.

leading to the enhanced production of A β -related peptides. Since expression of APP and its processing enzymes were increased in a subset of reactive astrocytes, it is likely that A β peptides are partly generated from these astrocytes. To validate this notion, rat primary astrocytes were treated with 100 μ M

kainic acid for different durations (12 h–48 h) and then cellular/secretory A β levels were measured using ELISA. Our results showed that cellular levels of A β _{1–40} were significantly increased at 12 h and 24 h, whereas A β _{1–42} were increased only at 12 h after kainic acid treatment (Figure 8A). However,

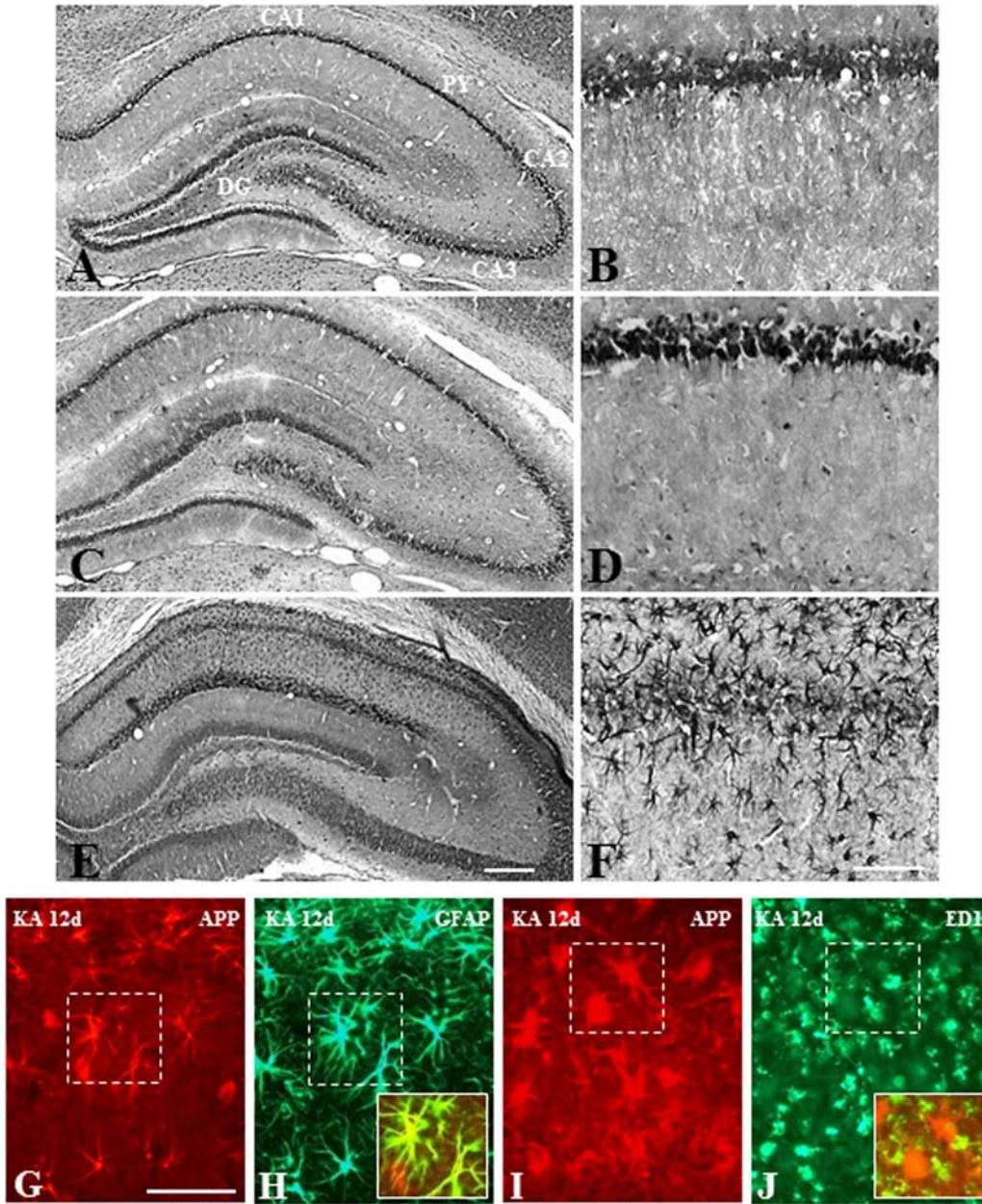


Figure 4. A-F; Photomicrographs depicting cellular distribution of APP in the hippocampus of saline-treated control (**A, B**) and following 12 h (**C, D**) and 12 day (**E, F**) kainic acid-treated rats at lower (**A, C, E**) and higher (**B, D, F**) magnifications. Note the expression of APP in pyramidal and granule cell layers in control rats, whereas after 12 day kainic

acid treatment APP is evident mostly in glial cells of the hippocampus. **G-J.** Double immunofluorescence photomicrographs showing a subset of GFAP-labeled astrocytes (**G, H**), but not ED1-labeled microglia (**I, J**), exhibit immunoreactive APP in 12 day kainic acid-treated rat. Py, pyramidal cell layer; DG, dentate gyrus. Scale bar, 100 μ M.

secretory levels of A β ₁₋₄₀ and A β ₁₋₄₂ were markedly increased with time following exposure to 100 μ M kainic acid (Figure 8B). Pre-treatment with the γ -secretase inhibitor DAPT attenuated enhanced levels of A β peptides, suggesting that an increased production/secretion of the peptides is triggered by kainic acid treatment (Figure 8C). In parallel, we observed that the viability of cultured astrocytes was not compromised after 48 h exposure to either 100 μ M kainic acid or 20 μ M DAPT (Figure 8D).

Kainic acid-induced toxicity of mixed neuron/astrocyte cultures protected by γ -secretase inhibitor

To determine the significance of A β peptides in kainic acid-induced toxicity, pure rat hippocampal cultured neurons were first exposed to kainic acid (50–200 μ M) for either 24 or 48 h, and then cell viability was assessed using MTT assay. As expected, viability of neurons was reduced significantly in most conditions, but it did not

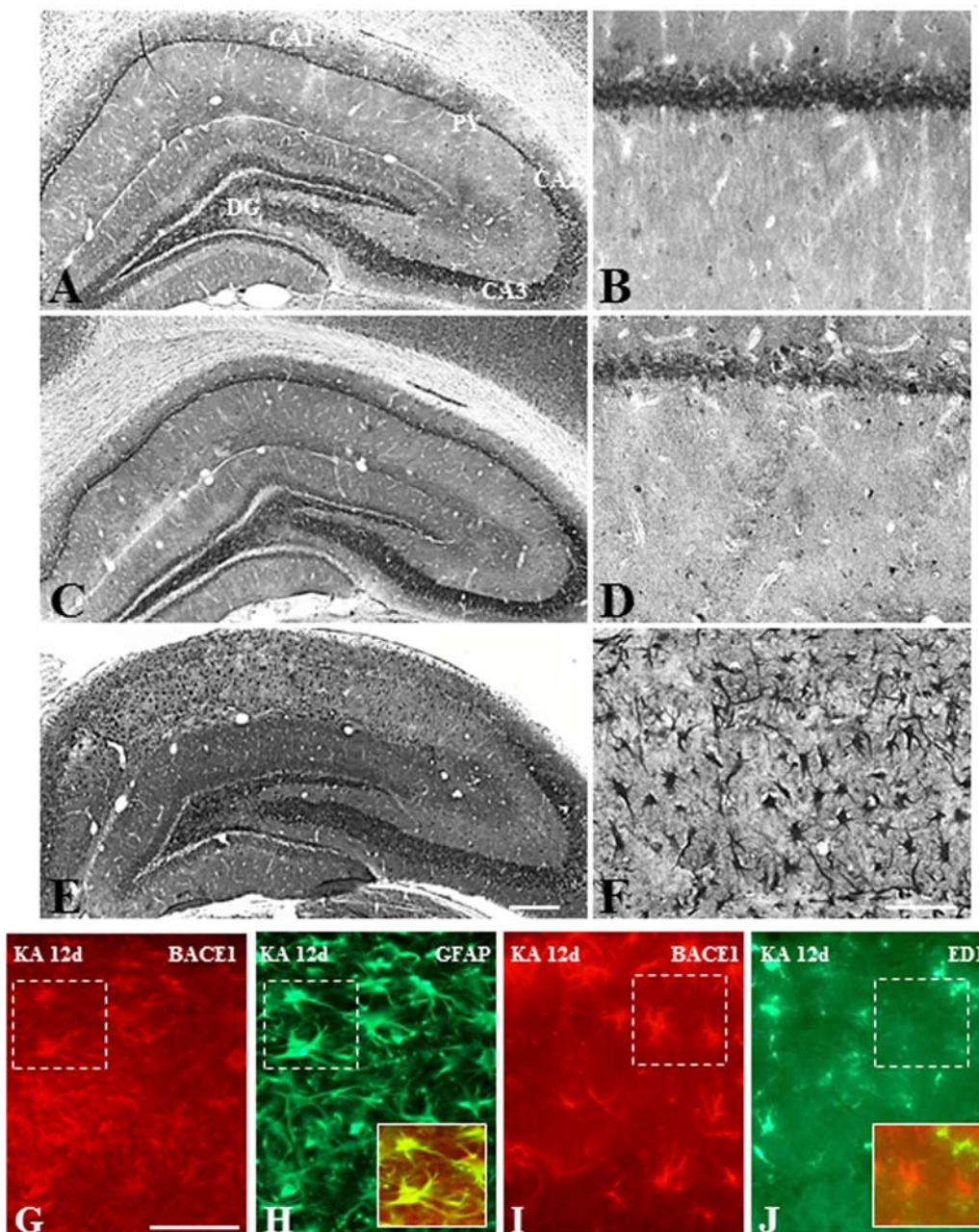


Figure 5. A–F. Photomicrographs depicting cellular distribution of BACE1 in the hippocampus of control (A, B) and following 12 h (C, D) and 12day (E, F) kainic acid-treated rats at lower (A, C, E) and higher (B, D, F) magnifications. Note the expression of BACE1 in pyramidal and granule cell layers in control rats, whereas after 12 day kainic acid

treatment BACE1 is evident mostly in glial cells of the hippocampus. **G–J.** Double immunofluorescence photomicrographs showing a subset of GFAP-labeled astrocytes (G, H), but not ED1-labeled microglia (I, J), exhibit immunoreactive BACE1 in 12 day kainic acid-treated rat. Py, pyramidal cell layer; DG, dentate gyrus. Scale bar, 100 μ M.

exhibit marked variation with either increasing concentrations or time following exposure to kainic acid (Figure 8E). Interestingly, treating mixed neuron/astrocyte co-cultures with 100 μ M kainic acid for 24 h was found to reduce cell viability significantly more than what was observed with pure neuronal cultures (Figure 8F). Since kainic acid did not affect the viability of cultured astrocytes (Figure 8D), we wanted to establish if A β peptides generated from

cultured astrocytes in response to this treatment may be involved in triggering neurotoxicity. Thus, we pretreated mixed neuron/astrocyte co-cultures with or without 20 μ M DAPT, and then exposed them to 100 μ M kainic acid for 24 h. Our results clearly showed that DAPT treatment can attenuate kainic acid-induced toxicity (Figure 8G), indicating a role for A β peptides in reducing viability of neurons.

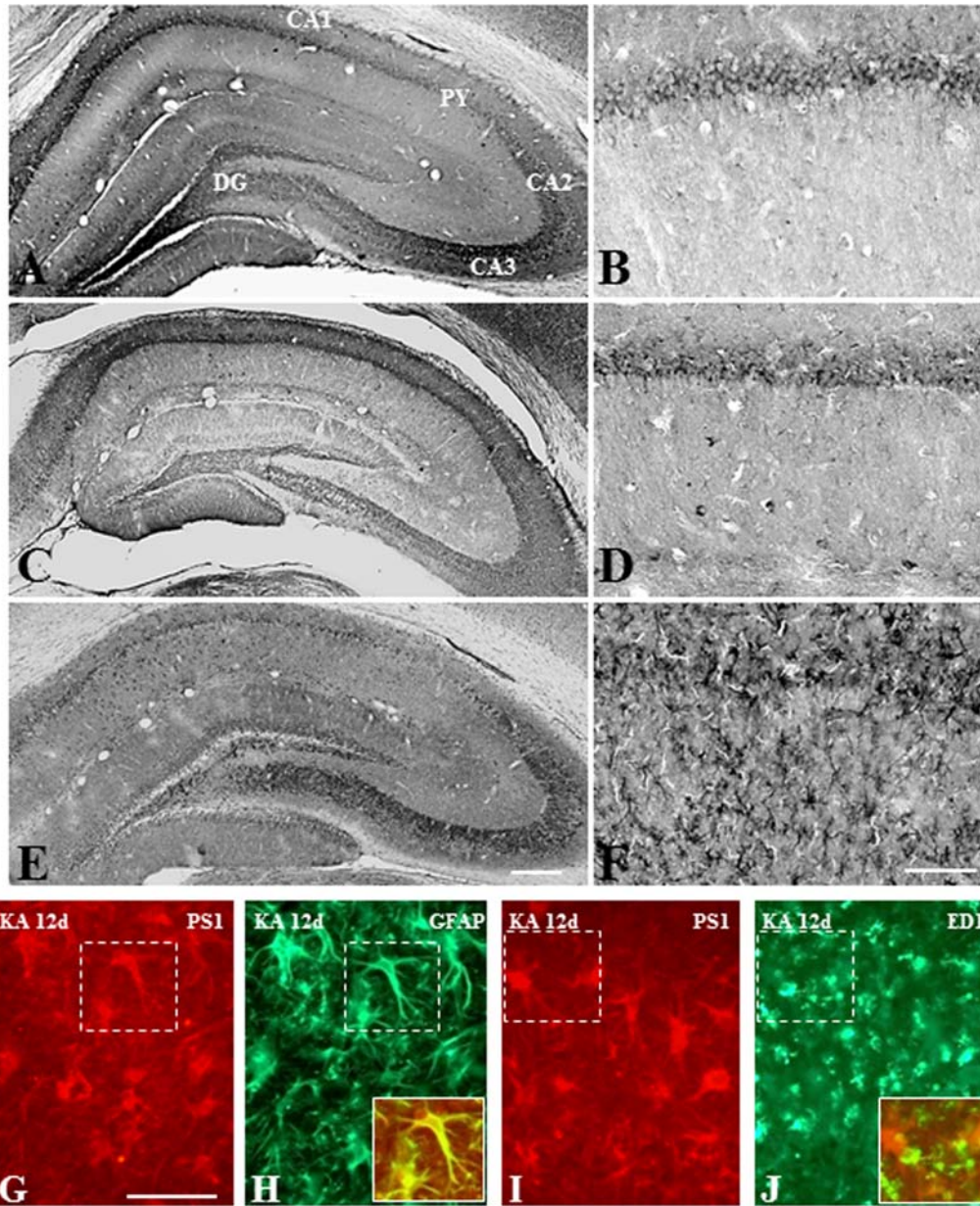


Figure 6. A–F. Photomicrographs depicting cellular distribution of PS1 in the hippocampus of saline-treated control (A, B) and following 12 h (C, D) and 12 day (E, F) kainic acid-treated rats at lower (A, C, E) and higher (B, D, F) magnifications. Note the expression of PS1 in pyramidal and granule cell layers in control rats, whereas after 12 day kainic acid

treatment PS1 is evident mostly in glial cells of the hippocampus. **G–J.** Double immunofluorescence photomicrographs showing a subset of GFAP-labeled astrocytes (G, H), but not ED1-labeled microglia (I, J), exhibit immunoreactive PS1 in 12 day kainic acid-treated rat. Py, pyramidal cell layer; DG, dentate gyrus. Scale bar, 100 μ M.

DISCUSSION

This study uses a combination of experimental approaches to show that increased production and levels of A β peptides in reactive astrocytes have a role in neurotoxicity triggered by the systemic administration of kainic acid. This is supported by data which show that (i) kainic acid-induced loss of hippocampal neurons is associated with higher expression of APP, BACE1 and γ -secretase components in a subset of activated astrocytes, (ii) activity of β - and γ -secretases are enhanced along with the

levels of APP-cleaved products including A β _{1–40} and A β _{1–42} in the hippocampus of kainic acid-treated rats, (iii) rat A β _{1–42}, like human A β _{1–42}, is toxic to cultured hippocampal neurons, (iv) exposing rat cultured astrocytes to kainic acid can lead to enhanced production/secretion of A β _{1–40}/A β _{1–42} without compromising their viability and (v) loss of rat cultured hippocampal neurons by kainic acid is enhanced in the presence of astrocytes, and can be attenuated by the γ -secretase inhibitor DAPT. Collectively, these results suggest that activated astrocytes, by way

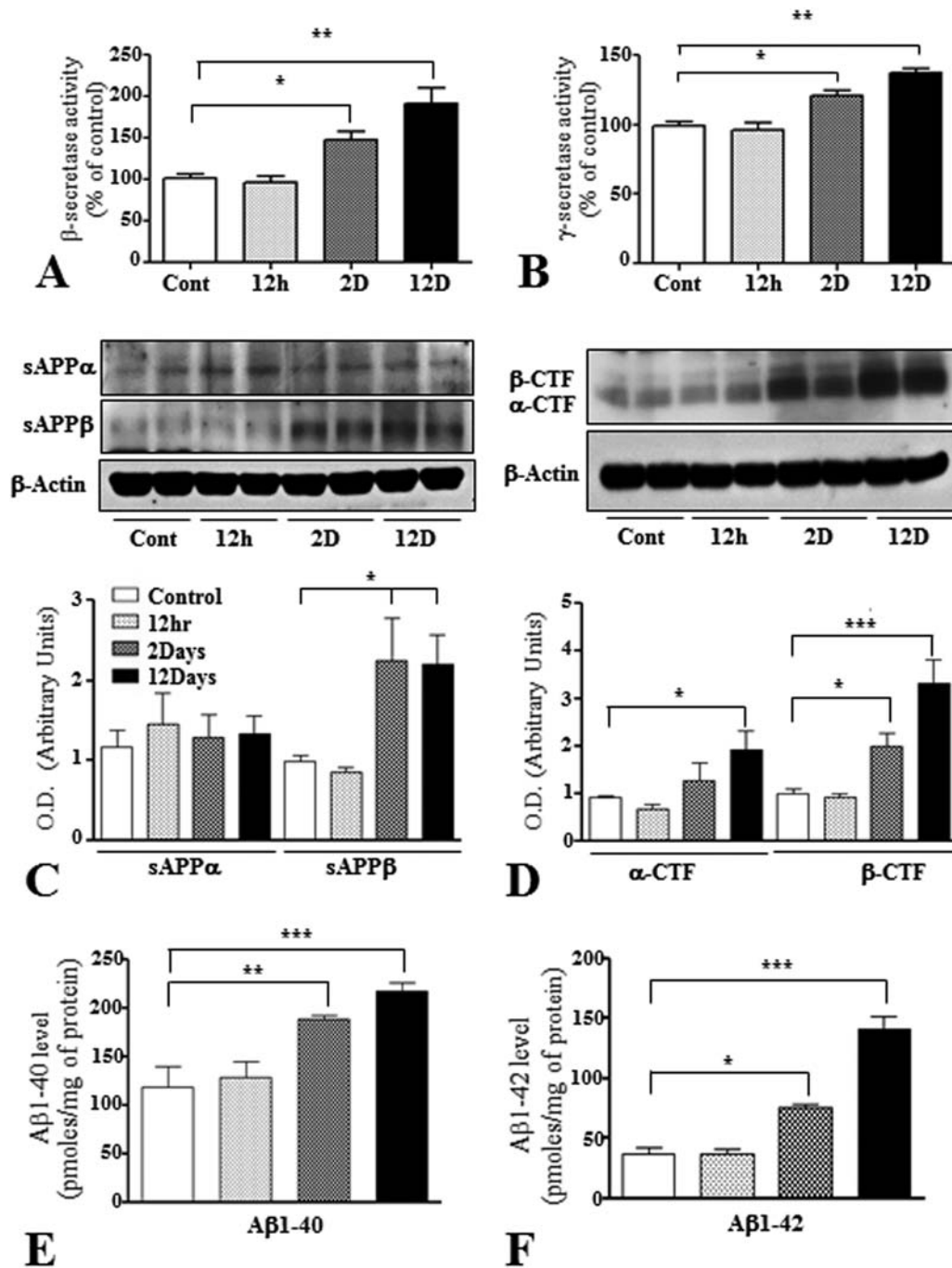


Figure 7. A and B. Histograms showing the activity of β- (A) and γ- (B) secretases in the hippocampus of control and kainic acid-treated rats. Note the relative increase in the activity of both secretases following systemic administration of kainic acid compared to control rats. **C and D.** Representative immunoblots and histograms showing levels of sAPPα and sAPPβ (C), α-CTF and β-CTF (D) in the hippocampus of control and kainic acid-treated rats. Note the relative increase in the levels of sAPPβ, α-CTF and β-CTF, but not sAPPα, following

administration of kainic acid compared to control rats. **E and F.** Histograms showing the quantification of rat Aβ₁₋₄₀ (E) and Aβ₁₋₄₂ (F), as detected by ELISA, in the hippocampus of control and kainic acid-treated rats. Note the significant increase in the levels of both Aβ peptides following kainic acid administration compared to control rats. All blots were re-probed with anti-β-actin to monitor protein loading. Values represent means ± SEM from 4–8 rats. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

of heightened APP expression and the increased production of Aβ peptides, play a critical role in reducing viability of neurons triggered by kainic acid. Since kainic acid treatment can lead to

neuropathological changes resembling those observed in human TLE, it is possible that APP/Aβ peptides derived from astrocytes may also have a role in TLE pathogenesis.

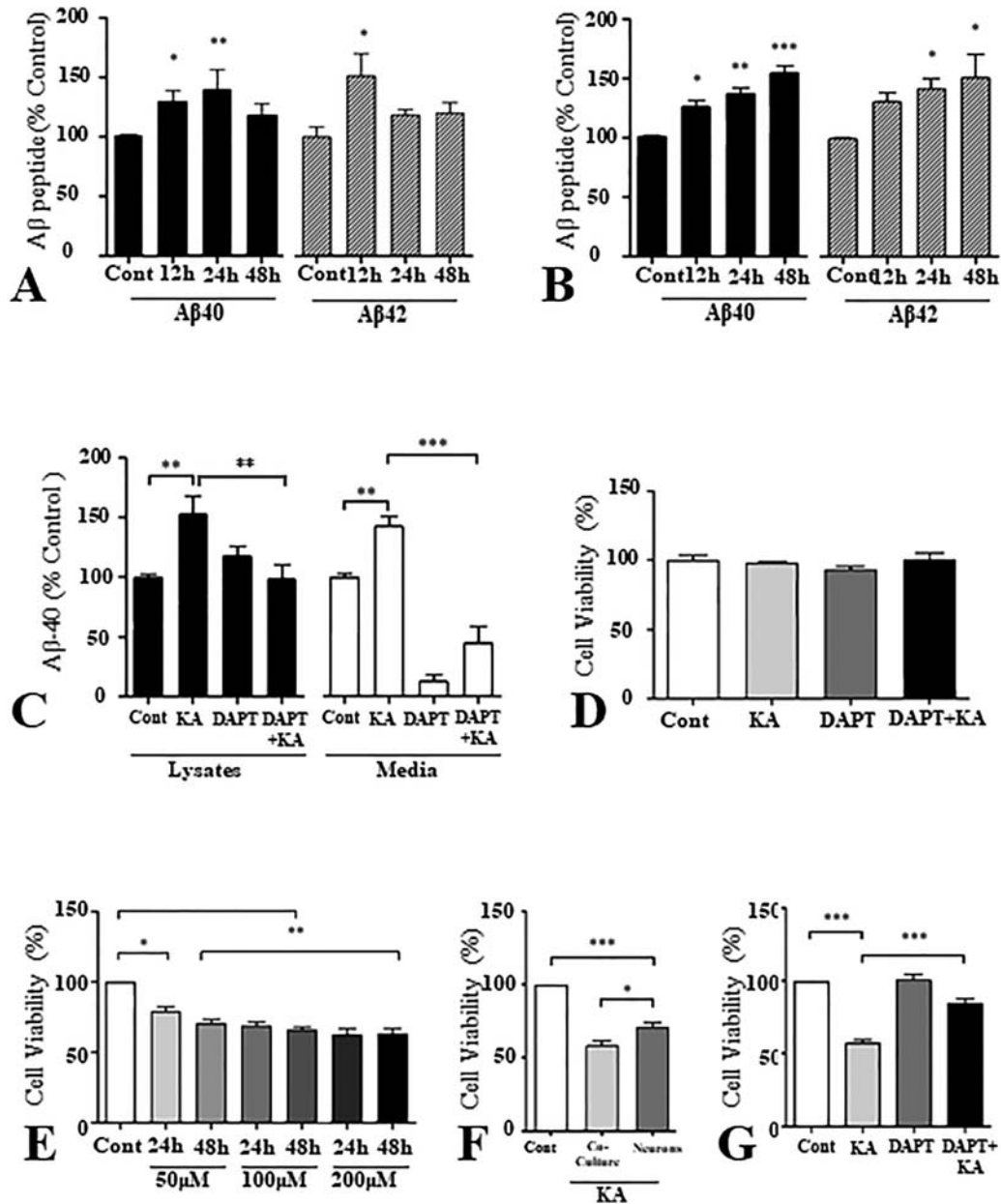


Figure 8. A and B. Histograms showing the alteration in A β ₁₋₄₀ and A β ₁₋₄₂ levels in cell lysates (A) and media (B) of rat primary astrocytes treated with 100 μ M of kainic acid over 12 h, 24 h and 48 h relative to control (Cont). Both A β ₁₋₄₀ and A β ₁₋₄₂ show a significant increase in the cell lysates as well as in the media following kainic acid treatment. **C.** Histogram depicting the levels of A β ₁₋₄₀ in primary rat astrocyte cultures treated with either kainic acid, γ -secretase inhibitor DAPT, or a combination of the two (DAPT + KA) relative to control. Note that DAPT drastically reverses the increase in A β ₁₋₄₀ levels triggered by kainic acid and this effect is especially prominent in the conditioned media. **D.** Histogram showing no change in the viability of cultured astrocytes following

treatment with kainic acid, DAPT or DAPT + kainic acid, as measured by an MTT assay. **E.** Histogram showing the effect of kainic acid on the viability of rat primary hippocampal cultured neurons as assessed by an MTT assay. Kainic acid was found to be toxic to neurons in relation to control. **F.** Histogram showing viability of kainic acid-treated neuron/astrocyte co-cultures and pure neuronal cultures compared to control. **G.** Histogram showing the viability of neuron/astrocyte co-cultures in relation to control following treatment with kainic acid, DAPT and DAPT + kainic acid. Kainic acid-induced death of neurons was attenuated by DAPT pre-treatment. All cultured data represent means \pm SEM from 3-4 independent experiments. * P < 0.05, ** P < 0.01, *** P < 0.001.

Loss of neurons in kainic acid-treated rats

Consistent with earlier studies, we observed that systemic administration of kainic acid can induce loss of neurons, along with glial

hypertrophy and proliferation in the hippocampus (8, 45, 80, 84). These effects are largely mediated by kainate receptor-induced glutamatergic currents that trigger an influx of Ca⁺² and subsequent

activation of downstream signaling cascades including ERK1/2, calpain and caspase as observed in the present study (4, 10, 34, 82, 93). Since neuronal vulnerability to excitotoxicity can be enhanced by A β peptides (53), and glutamate receptor activation can regulate amyloidogenic processing of APP (13, 43, 64), it is of critical relevance to determine if A β peptides may have a role in reducing viability of neurons following kainic acid administration. This will not only refine the molecular pathways associated with seizure-induced neuronal death mechanisms, but also may provide novel treatment targets for patients with TLE.

Influence of kainic acid administration on APP and its processing enzymes

Some earlier studies have shown that kainic acid can trigger APP expression (72, 91), but its significance in the development of pathological features and/or loss of neurons remains unclear. The present study clearly demonstrates that systemic administration of kainic acid increases not only the levels of APP, but also the expression/levels as well as activity of both BACE1 and the γ -secretase complex. These alterations, which appeared first at 2 days and increased following 12 days post-treatment, are accompanied by a time-dependent increase in APP cleaved intermediates including sAPP β and β -CTF, and most importantly, A β ₁₋₄₀ and A β ₁₋₄₂ peptides. Collectively, these results suggest that increased levels and amyloidogenic processing of APP may have a critical role in the development of TLE pathology. Interestingly, certain neurodevelopmental disorders such as Fragile X syndrome (FXS) and autism spectrum disorders (ASD), unlike TLE, have been shown to be associated with increased processing of APP via non-amyloidogenic pathway. While FXS is the most common form of inherited intellectual disability, ASD is characterized by persistent deficits in social communication/interactions and stereotypic repetitive behaviors (27, 88). Brain abnormalities common to FXS and ASD include deranged cortical pyramidal dendritic spines and increased disruption of white as well as grey matters contributing to macrocephaly (32, 33, 60). Pivotal work from two independent laboratories have demonstrated significantly elevated levels of sAPP α with a concomitant reduction in sAPP β and A β levels in plasma and brain tissue samples of children with ASD (59, 60, 74). Similarly, it has been shown that levels of APP and sAPP α are increased in the plasma as well as brain samples of individuals with FXS (60, 88). Given the neurotrophic roles of sAPP α , it has been suggested that increased non-amyloidogenic processing of APP may result in macrocephaly associated with FXS and ASD, whereas enhanced processing of APP via amyloidogenic pathway may be associated with neurodegeneration observed in AD and TLE (41, 88).

Under normal conditions, APP, BACE1 and γ -secretase components are usually localized in neurons of the brain, but not in glial cells (9, 37, 71, 76). The present study showed that a subset of reactive astrocytes, which increased gradually with the progression of disease pathology (10, 25, 45, 90), express APP and its processing enzymes in the hippocampus of kainic acid-treated rats. In fact, some earlier studies have reported that expression of APP, BACE1, PS1 and/or nicastrin can be induced in activated astrocytes of the brain following a variety of surgical/pathological lesions, such as cerebral ischemia, traumatic brain injury, excitotoxicity and cholesterol sequestration within the endosomal compartments (5, 7, 38,

54, 56, 72, 94). There is also evidence that activated astrocytes located in proximity to A β -containing neuritic plaques in AD brains and in mutant APP transgenic mice express higher levels of APP and/or its processing enzymes (31, 55, 63, 73). Although the underlying mechanisms behind this occurrence remain unclear, it is possible that factors released from activated microglia or damaged neurons trigger astrocytic expression of APP and its processing enzymes (62).

Role of A β peptides in kainic acid-treated rats

The administration of A β peptides has been implicated in the induction and propagation of seizures by possibly increasing extracellular glutamate levels caused by enhanced release and decreased uptake of glutamate (24, 36, 51, 66). The present study clearly shows that kainic acid can trigger an increased production and secretion of A β ₁₋₄₀/A β ₁₋₄₂ peptides, most likely in reactive astrocytes, which may partly be involved in the induction/propagation of seizures. In parallel, A β peptides may also have a role in the kainic acid-driven loss of neurons. This is supported by three distinct lines of evidence—first, our results reveal that rat A β peptides, as observed earlier (15), can be toxic to neurons. Second, we demonstrated that kainic acid treatment can enhance cellular/secretory levels of both A β ₁₋₄₀ and A β ₁₋₄₂ from rat primary astrocytes without affecting their viability. Finally, using neuronal and neuronal/astrocyte mixed cultures we showed that the presence of astrocytes exacerbates the neuronal toxicity of kainic acid, which can be ameliorated by pretreatment with the γ -secretase inhibitor DAPT. Thus, increased levels of A β derived from astrocytes may directly be involved in mediating toxicity induced by kainic acid. However, the underlying mechanism by which astrocytic A β decreased neuronal vulnerability remains unclear. It is possible that A β secreted from astrocytes following kainic acid treatment can act directly on neurons to decrease their vulnerability. Alternatively, A β peptide secreted from kainic acid-treated astrocytes may act to increase the levels/processing of APP in neurons leading to enhanced intraneuronal A β level (57) which can then decrease neuronal vulnerability.

Significance of A β -related peptides in TLE

Although A β -containing plaques and increased levels of APP are apparent in the brain of some TLE patients (26, 46, 69, 70), the significance of these peptides in disease pathogenesis remain unclear. The predominant pathological feature associated with TLE is hippocampal sclerosis, which is characterized by atrophy, induration, gliosis, synaptic reorganization and loss of neurons in CA1, CA3 and the dentate hilar regions (12, 47, 77, 80). There is evidence that excitotoxic glutamatergic transmission resulting from neuronal hyperactivity has a role in triggering recurrent seizures and death of neurons in the sclerotic hippocampus. More recently, a number of studies have shown that activated astrocytes, by regulating the uptake and release of glutamate, can directly contribute to the generation of seizures and loss of neurons in the hippocampus (17, 23, 25). Our results, conversely, demonstrate that levels of A β -related peptides, which increase gradually in kainic acid-treated rats may originate, at least in part, from a subset of activated astrocytes exhibiting enhanced expression of APP and its processing enzymes. Since A β peptides can elevate extracellular glutamate

levels by potentiating its release (36, 61, 66) and inhibiting its uptake by astrocytes (24, 30), our data indicate a role for A β in the development of seizures and subsequent loss of neurons in TLE brains. This is supported by the evidence that (i) A β peptide can directly induce neuronal hyperexcitability and trigger epilepsy (51), (ii) transgenic mice overexpressing A β peptide exhibit spontaneous as well as induced seizures more frequently than wild-type mice (20, 58, 87), (iii) the prevalence of seizures is higher in AD cases than in control populations (2, 14, 42, 67) and the antiepileptic drugs can be able to partially reverse AD-related pathology (6, 65, 92, 95) and (iv) kainic acid-induced neurotoxicity, as apparent from our results, can be attenuated by inhibition of A β synthesis. Additionally, it is reported that immunization of mutant APP transgenic mice with A β peptide protects them from seizures (52). These results, taken together, not only highlight the significance of astrocytic A β in TLE pathology, but also raise the possibility that lowering A β levels may be beneficial in attenuating seizures and neurodegeneration.

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REFERENCES

- Alley GM, Bailey JA, Chen D, Ray B, Puli LK, Tanila H *et al* (2010) Memantine lowers amyloid-beta peptide levels in neuronal cultures and in APP/PS1 transgenic mice. *J Neurosci Res* **88**:143–154.
- Amatniek JC, Hauser WA, DelCastillo-Castaneda C, Jacobs DM, Marder K, Bell K *et al* (2006) Incidence and predictors of seizures in patients with Alzheimer's disease. *Epilepsia* **47**:867–872.
- Andrew RJ, Kellett KA, Thinakaran G, Hooper NM (2016) A greek tragedy: the growing complexity of Alzheimer amyloid precursor protein proteolysis. *J Biol Chem* **291**:19235–19244.
- Araújo IM, Gil JM, Carreira BP, Mohapel P, Petersen A, Pinheiro PS *et al* (2008) Calpain activation is involved in early caspase-independent neurodegeneration in the hippocampus following status epilepticus. *J Neurochem* **105**:666–676.
- Avila-Muñoz E, Arias C (2015) Cholesterol-induced astrocyte activation is associated with increased amyloid precursor protein expression and processing. *Glia* **63**:2010–2022.
- Bakker A, Krauss GL, Albert MS, Speck CL, Jones LR, Stark CE *et al* (2012) Reduction of hippocampal hyperactivity improves cognition in amnesic mild cognitive impairment. *Neuron* **74**:467–474.
- Banati RB, Gehrmann J, Wiessner C, Hossmann KA, Kreutzberg GW (1995) Glial expression of the β -amyloid precursor protein (APP) in global ischemia. *J Cereb Blood Flow Metab* **15**:647–654.
- Banerjee M, Sasse A, Wang Y, Maulik M, Kar S (2015) Increased levels and activity of cathepsins B and D in kainate-induced toxicity. *Neuroscience* **284**:360–373.
- Beeson JG, Shelton ER, Chan HW, Gage FH (1994) Differential distribution of amyloid protein precursor immunoreactivity in the rat brain studied by using five different antibodies. *J Comp Neurol* **342**:78–96.
- Ben-Ari Y, Cossart R (2000) Kainate, a double agent that generates seizures: two decades of progress. *Trends Neurosci* **23**:580–587.
- Bi X, Chang V, Siman R, Tocco G, Baudry M (1996) Regional distribution and time-course of calpain activation following kainate-induced seizure activity in adult rat brain. *Brain Res* **726**:98–108.
- Blümcke I, Coras R, Miyata H, Ozkara C (2012) Defining clinico-neuropathological subtypes of mesial temporal lobe epilepsy with hippocampal sclerosis. *Brain Pathol* **22**:402–411.
- Bordji K, Becerril-Ortega J, Nicole O, Buisson A (2010) Activation of extrasynaptic, but not synaptic, NMDA receptors modifies amyloid precursor protein expression pattern and increases amyloid- β production. *J Neurosci* **30**:15927–15942.
- Born HA (2015) Seizures in Alzheimer's disease. *Neuroscience* **286**:251–263.
- Boyd-Kimball D, Sultana R, Mohmmad-Abdul H, Butterfield DA (2004) Rodent A β (1–42) exhibits oxidative stress properties similar to those of human A β (1–42): implications for proposed mechanisms of toxicity. *J Alzheimers Dis* **6**:515–525.
- Chishti MA, Yang DS, Janus C, Strome R, Horne P, Loukides J *et al* (2001) Early-onset amyloid deposition in transgenic mice expressing a double mutant form of APP695. *J Biol Chem* **276**:21562–21570.
- Coulter DA, Steinhäuser C (2015) Role of astrocytes in epilepsy. *Cold Spring Harb Perspect Med* **5**:a022434.
- Crépel V, Mulle C (2015) Physiopathology of kainate receptors in epilepsy. *Curr Opin Pharmacol* **20**:83–88.
- de Lanerolle NC, Lee TS, Spencer DD (2010) Astrocytes and epilepsy. *Neurotherapeutics* **7**:424–438.
- Del Vecchio RA, Gold LH, Novick SJ, Wong G, Hyde LA (2004) Increased seizure threshold and severity in young transgenic CRND8 mice. *Neurosci Lett* **367**:164–167.
- De Strooper B, Karran E (2016) The cellular phase of Alzheimer's disease. *Cell* **164**:603–615.
- Ding S, Fellin T, Zhu Y, Lee SY, Auberson YP, Meaney DF *et al* (2007) Enhanced astrocytic Ca²⁺ signals contribute to neuronal excitotoxicity after status epilepticus. *J Neurosci* **27**:10674–10684.
- Eid T, Williamson A, Lee TSW, Petroff OA, de Lanerolle NC (2008) Glutamate and astrocytes - key players in human mesial temporal lobe epilepsy?. *Epilepsia* **49**:42–52.
- Fernandez-Tome P, Brera B, Arevalo M, de Ceballos ML (2004) β -amyloid25–35 inhibits glutamate uptake in cultured neurons and astrocytes: modulation of uptake as a survival mechanism. *Neurobiol Dis* **15**:580–589.
- Gibbons MB, Smeal RM, Takahashi DK, Vargas JR, Wilcox KS (2013) Contributions of astrocytes to epileptogenesis following status epilepticus: opportunities for preventive therapy?. *Neurochem Int* **63**:660–669.
- Gouras GK, Relkin NR, Sweeney D, Munoz DG, Mackenzie IR, Gandy S (1997) Increased apolipoprotein E epsilon 4 in epilepsy with senile plaques. *Ann Neurol* **41**:402–404.
- Ha S, Sohn IJ, Kim N, Sim HJ, Cheon KA (2015) Characteristics of brains in autism spectrum disorder: structure, function and connectivity across the lifespan. *Exp Neurol* **24**:273–284.
- Haass C, Kaether C, Thinakaran G, Sisodia S (2012) Trafficking and proteolytic processing of APP. *Cold Spring Harb Perspect Med* **2**:a006270.
- Hadera MG, Eloquent H, Jaradat S, Nehlig A, Sonnewald U (2015) Astrocyte-neuronal interactions in epileptogenesis. *J Neurosci Res* **93**:1157–1164.
- Harris ME, Wang Y, Pedigo NW, Hensley K, Butterfield DA, Camey JM (2002) Amyloid β peptide (25–35) inhibits Na⁺-dependent glutamate uptake in rat hippocampal astrocyte cultures. *J Neurochem* **67**:277–286.

31. Hartlage-Rubsamen M, Zeitschel U, Apelt J, Gartner U, Franke H, Stahl T *et al* (2003) Astrocytic expression of the Alzheimer's disease β -secretase (BACE1) is stimulus-dependent. *Glia* **41**:169–179.
32. Hutsler JJ, Zhang H (2010) Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. *Brain Res* **1309**:83–94.
33. Irwin SA, Galvez R, Greenough WT (2000) Dendritic spine structural anomalies in fragile-X mental retardation syndrome. *Cereb Cortex* **10**:1038–1044.
34. Jiang W, Van Cleemput J, Sheerin AH, Ji SP, Zhang Y, Saucier DM *et al* (2005) Involvement of extracellular regulated kinase and p38 kinase in hippocampal seizure tolerance. *J Neurosci Res* **81**:581–588.
35. Kar S, Seto D, Doré S, Chabot JG, Quirion R (1997) Systemic administration of kainic acid induces selective time dependent decrease in [¹²⁵I]insulin-like growth factor I, [¹²⁵I]insulin-like growth factor II and [¹²⁵I]insulin receptor binding sites in adult rat hippocampal formation. *Neuroscience* **80**:1041–1055.
36. Kabogo D, Rauw G, Amritraj A, Baker G, Kar S (2010) β -amyloid-related peptides potentiate K⁺-evoked glutamate release from adult rat hippocampal slices. *Neurobiol Aging* **31**:1164–1172.
37. Kodam A, Vetrivel KS, Thinakaran G, Kar S (2008) Cellular distribution of gamma-secretase subunit nicastrin in the developing and adult rat brains. *Neurobiol Aging* **29**:724–738.
38. Kodam A, Maulik M, Peake K, Amritraj A, Vetrivel K, Thinakaran G *et al* (2010) Altered levels and distribution of APP and its processing enzymes in Niemann-Pick Type C1-deficient mouse brains. *Glia* **58**:1267–1281.
39. Kaye R, Lasagna-Reeves CA (2013) Molecular mechanisms of amyloid oligomers toxicity. *J Alzheimers Dis* **33**:S67–S78.
40. Klyubin I, Wang Q, Reed MN, Irving EA, Upton N, Hofmeister J *et al* (2011) Protection against A β -mediated rapid disruption of synaptic plasticity and memory by memantine. *Neurobiol Aging* **32**:614–623.
41. Lahiri DK, Sokol DK, Erickson C, Ray B, Ho CY, Maloney B (2013) Autism as early neurodevelopmental disorder: evidence for an SAPP α -mediated anabolic pathway. *Front Cell Neurosci* **7**:94.
42. Larner AJ (2010) Epileptic seizures in AD patients. *Neuromol Med* **12**:71–77.
43. Lesné S, Ali C, Gabriel C, Croci N, MacKenzie ET, Glabe CG *et al* (2005) NMDA receptor activation inhibits γ -secretase and promotes neuronal amyloid β production. *J Neurosci* **25**:9367–9377.
44. Lévesque M, Avoli M (2013) The kainic acid model of temporal lobe epilepsy. *Neurosci Biobehav Rev* **37**:2887–2899.
45. Lévesque M, Avoli M, Bernard C (2016) Animal models of temporal lobe epilepsy following systemic chemoconvulsant administration. *J Neurosci Methods* **260**:45–52.
46. Mackenzie IR, Miller LA (1994) Senile plaques in temporal lobe epilepsy. *Acta Neuropathol* **87**:504–510.
47. Majores M, Schoch S, Lie A, Becker AJ (2007) Molecular neuropathology of temporal lobe epilepsy: complementary approaches in animal models and human disease tissue. *Epilepsia* **48**:4–12.
48. Masters CL, Selkoe DJ (2012) Biochemistry of amyloid β -protein and amyloid deposits in Alzheimer disease. *Cold Spring Harb Perspect Med* **2**:a006262.
49. Maulik M, Peake K, Chung J, Wang Y, Vance JE, Kar S (2015) APP overexpression in absence of NPC1 exacerbates metabolism of amyloidogenic proteins of Alzheimer's disease. *Hum Mol Genetics* **24**:7132–7150.
50. Mehta A, Prabhakar M, Kumar P, Deshmukh R, Sharma PL (2013) Excitotoxicity: bridge to various triggers in neurodegenerative disorders. *Eur J Pharmacol* **698**:6–18.
51. Minkeviciene R, Rheims S, Dobszay MB, Zilberter M, Hartikainen J, Fülöp L *et al* (2009) Amyloid β -induced neuronal hyperexcitability triggers progressive epilepsy. *J Neurosci* **29**:3453–3462.
52. Mohajeri MH, Saini K, Schultz JG, Wollmer MA, Hock C, Nitsch RM (2002) Passive immunization against β -amyloid peptide protects central nervous system (CNS) neurons from increased vulnerability associated with an Alzheimer's disease-causing mutation. *J Biol Chem* **277**:33012–33017.
53. Morimoto K, Oda T (2003) Kainate exacerbates β -amyloid toxicity in rat hippocampus. *Neurosci Lett* **340**:242–244.
54. Nadler Y, Alexandrovich A, Grigoriadis N, Hartmann T, Rao KS, Shohami E, Stein R (2008) Increased expression of the gamma-secretase components presenilin-1 and nicastrin in activated astrocytes and microglia following traumatic brain injury. *Glia* **56**:552–567.
55. Nagele RG, D'Andrea MR, Lee H, Venkataraman V, Wang HY (2003) Astrocytes accumulate A β 42 and give rise to astrocytic amyloid plaques in Alzheimer disease brains. *Brain Res* **971**:197–209.
56. Nihashi T, Inao S, Kajita Y, Kawai T, Sugimoto T, Niwa M *et al* (2001) Expression and distribution of beta amyloid precursor protein and beta amyloid peptide in reactive astrocytes after transient middle cerebral artery occlusion. *Acta Neurochir (Wien)* **143**:287–295.
57. Ourdev D, Foroutanpay BV, Wang Y, Kar S (2015) The effect of oligomers A β _{1–42} on APP processing and A β _{1–40} generation in cultured U373 astrocytes. *Neurodegener Dis* **15**:361–368.
58. Palop JJ, Chin J, Roberson ED, Wang J, Thwin MT, Bien-Ly N *et al* (2007) Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron* **55**:697–711.
59. Ray B, Long JM, Sokol DK, Lahiri DK (2011) Increased secreted amyloid precursor protein-a (sAPP α) in severe autism: proposal of a specific, anabolic pathway and putative biomarker. *PLoS One* **6**:e20405.
60. Ray B, Sokol DK, Maloney B, Lahiri DK (2016) Finding novel distinctions between the sAPP α -mediated anabolic biochemical pathways in Autism Spectrum Disorder and Fragile X Syndrome plasma and brain tissue. *Sci Rep* **6**:26052.
61. Revett TJ, Baker GB, Jhamandas J, Kar S (2013) The glutamate system, amyloid β peptides and tau protein: functional interrelationships and relevance to Alzheimer's disease pathology. *J Psychiat Neurosci* **38**:6–23.
62. Rossner S, Lange-Dohna C, Zeitschel U, Perez-Polo JR (2005) Alzheimer's disease β -secretase BACE1 is not a neuron-specific enzyme. *J Neurochem* **92**:226–234.
63. Rossner S, Apelt J, Schliebs R, Perez-Polo JR, Bigl V (2001) Neuronal and glial β -secretase (BACE) protein expression in transgenic Tg2576 mice with amyloid plaque pathology. *J Neurosci Res* **64**:437–446.
64. Rush T, Buisson A (2014) Reciprocal disruption of neuronal signaling and A β production mediated by extrasynaptic NMDA receptors: a downward spiral. *Cell Tissue Res* **356**:279–286.
65. Sanchez PE, Zhu L, Verret L, Vessel KA, Orr AG, Cirrito JR *et al* (2012) Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. *Proc Natl Acad Sci USA* **109**:E2895–E2903.
66. Sanz-Blasco S, Piña-Crespo JC, Zhang X, McKercher SR, Lipton SA (2016) Levetiracetam inhibits oligomeric A β -induced glutamate release from human astrocytes. *Neuroreport* **27**:705–709.
67. Scharfman HE (2012) Untangling Alzheimer's disease and epilepsy. *Epilepsy Curr* **12**:178–183.
68. Schmued LC, Stowers CC, Scallet AC, Xu L (2005) Fluoro-Jade C results in ultrahigh resolution and contrast labeling of degenerating neurons. *Brain Res* **1035**:24–31.
69. Sheng JG, Boop FA, Mrak RE, Griffin WS (1994) Increased neuronal beta-amyloid precursor protein expression in human temporal lobe epilepsy: association with interleukin-1 alpha immunoreactivity. *J Neurochem* **63**:1872–1879.
70. Sima X, Xu J, Li J, Zhong W, You C (2014) Expression of β -amyloid precursor protein in refractory epilepsy. *Mol Med Rep* **9**:1242–1248.

71. Siman R, Salidas S (2004) Gamma-secretase subunit composition and distribution in the presenilin wild-type and mutant mouse brain. *Neuroscience* **129**:615–628.
72. Siman R, Card JP, Nelson RB, Davis LG (1989) Expression of β -amyloid precursor protein in reactive astrocytes following neuronal damage. *Neuron* **3**:275–285.
73. Simpson JE, Ince PG, Lace G, Forster G, Shaw PJ, Matthews F *et al* (2010) Astrocyte phenotype in relation to Alzheimer-type pathology in the aging brain. *Neurobiol Aging* **31**:578–590.
74. Sokol DK, Chen D, Farlow MR, Dunn DW, Maloney B, Zimmer JA, Lahiri DK (2006) High levels of Alzheimer beta-amyloid precursor protein (APP) in children with severely autistic behavior and aggression. *J Child Neurol* **21**:444–449.
75. Song MS, Rauw G, Baker GB, Kar S (2008) Memantine protects rat cortical cultured neurons against β -amyloid-induced toxicity by attenuating tau phosphorylation. *Eur J Neurosci* **28**:1989–2002.
76. Sun A, Koelsch G, Tang J, Bing G (2002) Localization of beta-secretase memapsin 2 in the brain of Alzheimer's patients and normal aged controls. *Exp Neurol* **175**:10–22.
77. Thom M (2014) Hippocampal sclerosis in epilepsy: a neuropathology review. *Neuropathol Appl Neurobiol* **40**:520–543.
78. Tremblay R, Chakravarthy B, Hewitt K, Tauskela J, Morley P, Atkinson T, Durkin JP (2000) Transient NMDA receptor inactivation provides long-term protection to cultured cortical neurons from a variety of death signals. *J Neurosci* **20**:7183–7192.
79. Vetrivel KS, Kodam A, Gong P, Chen Y, Parent AT, Kar S, Thinakaran G (2008) Localization and regional distribution of p23/TMP21 in the brain. *Neurobiol Dis* **32**:37–49.
80. Vincent P, Mulle C (2009) Kainate receptors in epilepsy and excitotoxicity. *Neuroscience* **158**:309–323.
81. Wang Y, Han R, Liang ZQ, Wu JC, Zhang XD, Gu ZL, Qin ZH (2008) An autophagic mechanism is involved in apoptotic death of striatal neurons induced by the non-N-methyl-D-aspartate receptor agonist kainic acid. *Autophagy* **4**:214–226.
82. Wang Y, Qin ZH (2010) Molecular and cellular mechanisms of excitotoxic neuronal death. *Apoptosis* **15**:1382–1402.
83. Wang Y, Buggia-Prevot V, Zavorka ME, Bleackley RC, MacDonald RG, Thinakaran G, Kar S (2015) Overexpression of the insulin-like growth factor-II receptor increases β -amyloid production and affects cell viability. *Mol Cell Biol* **35**:2368–2384.
84. Wang Q, Yu S, Simonyi A, Sun GY, Sun AY (2005) Kainic acid-mediated excitotoxicity as a model for neurodegeneration. *Mol Neurobiol* **31**:3–15.
85. Waxman EA, Lynch DR (2005) N-methyl-D-aspartate receptor subtypes: multiple roles in excitotoxicity and neurological disease. *Neuroscientist* **11**:37–49.
86. Wei Z, Song MS, MacTavish D, Jhamandas JH, Kar S (2008) Role of calpain and caspase in β -amyloid-induced cell death in rat primary septal cultured neurons. *Neuropharmacology* **54**:721–733.
87. Westmark CJ, Westmark PR, Beard AM, Hildebrandt SM, Malter JS (2008) Seizure susceptibility and mortality in mice that overexpress amyloid precursor protein. *Int J Clin Exp Pathol* **1**:157–168.
88. Westmark CJ, Sokol DK, Maloney B, Lahiri DK (2016) Novel roles of amyloid-beta precursor protein metabolites in fragile X syndrome and autism. *Mol Psychiatry* **21**:1333–1341.
89. White HS (2002) Animal model of epileptogenesis. *Neurology* **59**:S7–S14.
90. Wilcox KS, Gee JM, Gibbons MB, Tvrdik P, White JA (2015) Altered structure and function of astrocytes following status epilepticus. *Epilepsy Behav* **49**:17–19.
91. Willoughby DA, Johnson SA, Pasinetti GM, Tocco G, Najm I, Baudry M, Finch CE (1992) Amyloid precursor protein mRNA encoding the Kunitz protease inhibitor domain is increased by kainic acid-induced seizures in rat hippocampus. *Exp Neurol* **118**:332–339.
92. Xiao R (2016) Levetiracetam might act as an efficacious drug to attenuate cognitive deficits of Alzheimer's disease. *Curr Top Med Chem* **16**:565–573.
93. Yamagata Y, Nairn AC (2015) Contrasting features of ERK1/2 activity and synapsin I phosphorylation at the ERK1/2-dependent site in the rat brain in status epilepticus induced by kainic acid in vivo. *Brain Res* **1625**:314–323.
94. Yang H, Wang Y, Kar S (2017) U18666A-induced cholesterol sequestration and APP metabolism in rat primary astrocytes. *Glia* **65**:1728–1743.
95. Zhang MY, Zheng CY, Zou MM, Zhu JW, Zhang Y, Wang J *et al* (2014) Lamotrigine attenuates deficits in synaptic plasticity and accumulation of amyloid plaques in APP/PS1 transgenic mice. *Neurobiol Aging* **35**:2713–2725.
96. Zheng WH, Bastianetto S, Mennicken F, Ma W, Kar S (2002) Amyloid β peptide induces tau phosphorylation and neuronal degeneration in rat primary septal cultured neurons. *Neuroscience* **115**:201–211.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. A–L. Double immunofluorescence photomicrographs showing the localization of APP (A–D), BACE1 (E–H) and PS1 (I–L) with astrocyte marker GFAP and activated microglia marker ED1 in the hippocampal CA1 region of saline-treated control rats. Note the localization of APP, BACE1 and PS1 in neurons, GFAP in astrocytes but the absence of ED1 labeling due to lack of activated microglia in control brains. Scale bar, 100 μ M.

Figure S2. A–F. Photomicrographs depicting cellular distribution of nicastrin in the hippocampus of saline-treated control (A, B) and following 12 h (C, D) and 12 day (E, F) kainic acid-treated rats at lower (A, C, E) and higher (B, D, F) magnifications. Note the expression of nicastrin in pyramidal and granule cell layers in control rats, whereas after 12 day kainic acid treatment nicastrin is evident mostly in glial cells of the hippocampus. G–J. Double immunofluorescence photomicrographs showing a subset of GFAP-labeled astrocytes (G, H), but not ED1-labeled microglia (I, J), exhibit immunoreactive nicastrin in 12 day kainic acid-treated rats. Py, pyramidal cell layer; DG, dentate gyrus. Scale bar, 100 μ M.

Figure S3. A–F. Photomicrographs depicting cellular distribution of PEN2 in the hippocampus of saline-treated control (A, B) and following 12 h (C, D) and 12 day (E, F) kainic acid-treated rats at lower (A, C, E) and higher (B, D, F) magnifications. Note the expression of PEN2 in pyramidal and granule cell layers in control rats, whereas after 12 day kainic acid treatment PEN2 is evident mostly in glial cells of the hippocampus. G–J. Double immunofluorescence photomicrographs showing a subset of GFAP-labeled astrocytes (G, H), but not ED1-labeled microglia (I, J), exhibit immunoreactive PEN2 in 12 day kainic acid-treated rats. Py, pyramidal cell layer; DG, dentate gyrus. Scale bar, 100 μ M.

Figure S4. A–F. Photomicrographs depicting cellular distribution of A β 1 in the hippocampus of saline-treated control (A,

B) and following 12hr (C, D) and 12day (E, F) kainic acid-treated rats at lower (A, C, E) and higher (B, D, F) magnifications. Note the expression of APH1 in pyramidal and granule cell layers in control rats, whereas after 12 day kainic acid treatment APH1 is evident mostly in glial cells of the hippocampus. G–J. Double immunofluorescence photomicrographs showing a subset of GFAP-labeled astrocytes (G, H), but not ED1-labeled microglia (I, J), exhibit immunoreactive APH1 in 12 day kainic acid-treated rats. Py, pyramidal cell layer; DG, dentate gyrus. Scale bar, 100 μ M.

Figure S5. A–L. Double immunofluorescence photomicrographs showing the localization of nicastrin (A–D), PEN2 (E–H) and APH1 (I–L) with astrocyte marker GFAP and activated microglia marker ED1 in the hippocampal CA1 region of saline-treated control rats. Note the localization of nicastrin, PEN2 and APH1 in neurons, GFAP in astrocytes but the absence of ED1 labeling due to lack of activated microglia in control brains. Scale bar, 100 μ M.

Figure S6. A–C. Histograms showing the dose-dependent effects of 24 h exposure to different concentrations of human A β _{1–42} (A) and dose- as well as time-dependent effects of rat A β _{1–42} (B, C) on viability of hippocampal cultured neurons as revealed by MTT assay. Control cultures were treated with 10 μ M human or rat A β _{42–1} peptide. Note that human A β _{1–42} was found to be more toxic than equimolar concentration of rat A β _{1–42} on cultured neurons. D–G. Images from the live/dead assay depicting cell viability in control (D, E) and 20 μ M rat A β _{1–42}-treated (F, G) hippocampal cultured neurons. Calcein AM staining (green) represents intracellular esterase activity in living neurons, while EthD-1 (red) shows dead neurons with disintegrated plasma membrane. Note the toxic potency of rat A β _{1–42} compared to control cultures treated with rat A β _{42–1}. MTT data presented as % of control (means \pm SEM) were obtained from 3–5 separate experiments, each performed in triplicate. Cont, control. * $P < 0.05$ and ** $P < 0.001$ compared to control value. Scale bar, 50 μ m.