대한체질인류학회지 제 29 권 제 2 호
Korean J Phys Anthropol Vol. 29, No. 2 (2016) pp. 71~79
http://dx.doi.org/10.11637/kjpa.2016.29.2.71

Original Article

Kainic Acid Treatment Increases Ca^{2+}-mediated Neurotoxicity in the Mouse Hippocampus

Jung Eun Lee

Department of Thoracic and Cardiovascular Surgery, Gyeongsang National University Hospital, Institute of Health Sciences, Gyeongsang National University School of Medicine

(Received 13 May 2016, revised 21 June 2016, accepted 22 June 2016)

Abstract : Kainic acid (KA)-induced neuronal cell death is associated with intracellular Ca^{2+} influx. However, it is unknown whether Lyn/Btk pathway is involved in the Ca^{2+}-mediated neurotoxicity and neuronal death induced by KA. In the present study, we investigated the altered expression of Ca^{2+}-controlled proteins in KA-treated hippocampus. Mice were sacrificed at 24 h after KA (20 mg/kg) systemic injection. We conducted Electroencephalographic (EEG) recording and examined hippocampal alterations by Western blotting and immunostaining in control mice or KA-treated mice. EEG tests showed that KA-treated mice increased seizure frequency and severity compared with control mice during KA-induced seizures. We found that KA decreases hippocalcin and calpain-mediated proteolysis in the hippocampus. In particular, the phosphorylation of Lyn and Btk was increased in KA-treated hippocampus compared to those of control mice. Our findings identify tyrosine kinases such as Lyn/Btk as a critical regulator of Ca^{2+}-mediated neurotoxicity in KA-induced seizures.

Keywords : Kainic acid, Calcium, Lyn/Btk, Seizure, Hippocampus

Introduction

Systemic injection of a convulsive dose of kainic acid (KA), which is an analog of glutamate, results in pyramidal cell death in the CA1 and CA3 areas of the hippocampus [1-3]. Neuronal cell death from the overactivation of glutamate receptors is closely associated with abnormal calcium influx [4]. In addition, reactive gliosis and inflammatory cytokines contribute to calcium-mediated excitotoxicity and seizure severity, leading to neuronal cell death [5]. It is well known that enhanced calcium influx results in an enhanced inflammatory response and a detrimental seizure phenotype characterized by neuronal excitability and excitotoxicity [6]. However, despite many studies, the underlying mechanism involved in neuronal death following calcium-mediated excitotoxicity remains unclear.

KA causes an acute increase of intracellular Ca^{2+} levels in pyramidal neurons in the hippocampus, resulting in mitochondrial Ca^{2+} overload and release of cytochrome c [3,7]. The endoplasmic reticulum (ER) is also reported to contribute to Ca^{2+} overload [8]. For compensation of this excessive Ca^{2+} overload within intercellular space in the KA-treated hippocampus, it has been well known that Ca^{2+}-binding proteins such as parvalbumin, calretinin, and calbindin-D_{28k} have the capacity to buffer intracellular Ca^{2+} [9]. In particular, parvalbumin has a neuroprotective effect by prevention of increasing in intracellular Ca^{2+}

*This study was supported by the Gyeongsang National University Hospital Fund for Professors on Sabbatical Leave, 2015.
The author(s) declare that there are no conflicts of interest.
Correspondence to : Jung Eun Lee (Department of Thoracic and Cardiovascular Surgery, Gyeongsang National University Hospital, Gyeongsang National University)
E-mail : ilikerem@hanmail.net

ISSN 2287-626X (Online) · ISSN 1225-150X (Print)

concentration in ischemic brain injury [10]. Hippocalcin, which belongs to the Ca^{2+} binding protein family also plays an important role in the maintenance of Ca^{2+} homeostasis through the removal of excessive Ca^{2+} levels [11]. Previous studies have shown excitotoxic apoptosis to induce the release of pro-apoptotic molecules from mitochondria, including cytochrome c [12]. The release of cytochrome c induces the activation of a family of cysteine proteases [13]. Among these cysteine protease, calpain has been implicated in excitotoxic neuronal death [14]. Calpain cleaves cytoskeletal proteins and enzymes and may cause to morphological changes that mimic caspase-dependent apoptosis [15]. Because calpains are induced by an increase in cytoplasmic Ca^{2+}, calpains may specifically play an important role in morphological alterations during KA-induced neuronal cell death. Importanlty, tyrosine kinase activity is required for activation of the B cell receptor (BCR)-Ca^{2+} signalling pathway in mature B lymphocytes [16]. The BCR complex interacts with and is phosphorylated by one or more kinase family: Lyn, Fyn, and Btk [17]. In mast cells, the levels of intracellular Ca^{2+}, is regulated by 4-1BB signalling through the Lyn/Btk/Ca^{2+} pathway [18]. However, the underlying mechanism involved in KA-induced neuronal death following Lyn/Btk/Ca^{2+}-mediated excitotoxicity remains unclear.

In the present study, we sought to determine the regulation of Ca^{2+}-regulated buffering, proteolysis, and Lyn/Btk pathways in mice with KA-induced seizures. Our results provide valuable insight into the Ca^{2+}-mediated pathogenesis of seizure-induced neuronal death by introducing protein kinases as a key mediator of intracellular Ca^{2+} influx.

Materials and Methods

1. Animals

Male C57BL/6 mice (6 weeks old) purchased from Central Laboratory Animal Inc. (Seoul, South Korea) were maintained in the animal facility at Gyeongsang National University. All of the animal experiments were approved by the Institutional Board of Research at Gyeongsang National University (GNU) and performed in accordance with the National Institutes of Health guidelines for laboratory animal care. The University Animal Care Committee for Animal Research of GNU approved the study protocol (GNU-121221-M0108). Mice were individually housed with an alternating 12 h light/dark cycle and had free access to food pellets and tap water.

2. Behavioural observation of KA-induced seizures

KA (Abcam, Cambridge, MA, USA) was emulsified in 0.9% normal saline and mice (n = 10) were treated with an intraperitoneal (i.p.) injection of 20 mg/kg body weight. Mice in the control group (n = 10) received 0.9% normal saline. Seizure behaviour was monitored for 2 h after KA injection. Severity of seizures was categorized with a six-point seizure scale (I to VI) as previously described [5].

3. Electroencephalographic (EEG) recording

For recording of EEG after KA-induced seizures, we used a small animal radio-telemetry system from Data Sciences International (DSI, St. Paul, MN, USA). Brain electrical activity was monitored in freely mobile, control or KA-treated mice (n = 2 mice per group). Mice were anesthetized with zoletil (0.5 mg/kg; Virbac Laboratories, Carros, France). The surgical procedure employed was adapted from previous studies using similar equipment and as previously described [19]. Mice were singly housed thereafter and allowed to recover from surgery for 7 days prior to KA injection. EEG data were recorded with Neuro Score version 2.1 software (DSI). Off-line interictal spike sorting and frequency analysis were performed with an Auto seizure spike train detector. To discriminate abnormal interictal spikes from physiologically-occurring ones in epidural one-channel electroencephalograms, only the spikes that were followed by a wave were manually counted. EEG was monitored for 6 h.

4. Tissue preparation and Cresyl violet staining

For tissue analysis, mice (n = 4 mice per group) were anesthetized with zoletil (5 mg/kg, Virbac Laboratories) and then perfused transcardially with heparinized saline followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). After post-fixation in the same fixative for 6 h, the brains were sequentially immersed in 0.1 M PBS containing 15% sucrose, and then in PBS containing 30% sucrose at 4°C until they were completely submerged. The brains were cut into 40-μm thick coronal

sections. The sections stained with Cresyl violet were visualized with a BX51 light microscope (Olympus, Tokyo, Japan) and digital images were captured.

5. Protein extraction

After anaesthesia with zoletil, brains (n = 6 mice per group) were quickly removed from the skull and both hippocampi were dissected and frozen. Hippocampal whole cellular extracts were prepared as previously described [5]. Briefly, frozen hippocampi were individually transferred to sterile 1.5 mL microcentrifuge tubes containing 200 μL of tissue protein extraction reagent (Thermo Scientific, Waltham, MA, USA) and protease inhibitor cocktail (Sigma-Aldrich). Homogenized tissues were incubated for 10 min on ice and then sonicated. Samples were then centrifuged at 4°C for 30 min at 12,000 rpm. The supernatants were transferred to clean vials and samples were stored at −80°C.

6. Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL)

TUNEL analysis was performed to measure the degree of apoptosis in the tissues using an *in situ* cell death detection kit (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's protocol. The stained brain sections were visualized with a BX51-DSU microscope (Olympus) and digital images were captured.

7. Immunofluorescence (IF)

For immunofluorescence staining, the frozen free-floating brain sections were incubated with rabbit anti-hippocalcin antibodies (1 : 100, Abcam) at 4°C for 1 day. After washing three times with 0.1 M PBS, sections were incubated with Alexa Fluor 594-conjugated donkey anti-rabbit secondary antibody (Invitrogen, Carlsbad, CA, USA). For double immunofluorescence staining, the free-floating brain sections were incubated with rabbit anti-S100β (1 : 100, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-GFAP (1 : 500, Sigma-Aldrich, St. Louis, MO, USA) at 4°C for 2 days. After washing three times with 0.1 M PBS, sections were incubated with Alexa Fluor 594- and 488-conjugated donkey anti-rabbit and anti-mouse secondary antibodies (Invitrogen). Fluorescence was visualized with a BX51-DSU microscope (Olympus) and digital images were captured.

8. Western blot analysis

The protein concentration of each lysate was determined using a bicinchoninic acid kit (Pierce, Rockford, IL, USA), according to the manufacturer's protocol, using bovine serum albumin as a standard. Equal amounts of protein (15 μg) were separated by SDS-PAGE and transferred to PVDF Transfer membranes. The membranes were washed in Tris-buffered saline containing 0.5% Tween-20 and incubated with the primary antibodies; hippocalcin (1 : 1000, Abcam) S100β (1 : 1000, Santa Cruz Biotech), calpain2 (1 : 1000, Cell signaling Technology, Danvers, MA, USA), α-II spectrin (1 : 1000, Santa Cruz Biotech), tau (1 : 1000, Abcam), p-GSKβ (1 : 1000, Cell signaling), total GSKβ (1 : 1000, Cell signaling), p-Lyn (1 : 1000, Abcam),

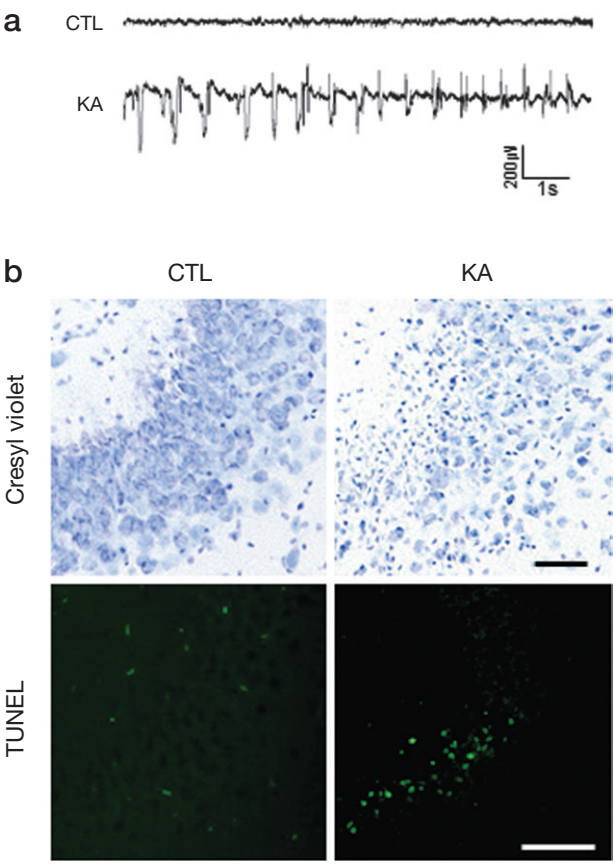

Fig. 1. Effects of KA treatment on seizure activity and hippocampal cell death. (a) EEG recording of a spontaneous seizure in control and KA-treated mice (n = 2 mice per group). (b) Cresyl violet-stained sections and TUNEL-positive cells from the CA3 region of control and KA-treated mice. Scale bar = 50 μm.

total Lyn (1 : 1000, Abcam), p-Btk (1 : 1000, Cell signaling), total Btk (1 : 1000, Santa Cruz Biotech). The samples were then incubated with their corresponding HRP-conjugated secondary antibodies. The enhanced chemiluminescence western blot analysis system (Amersham Pharmacia Biotech, Piscataway, NJ, USA) was used for detection. To normalize the protein levels, α-tubulin (1 : 50000, Sigma-Aldrich) or β-actin (1 : 30000, Sigma-Aldrich) was used as an internal control.

9. Statistical analysis

Student's t-test was used for comparisons of two groups. Values are expressed as the mean ± SEM. A P value < 0.05 was considered as statistically significant.

Results

1. Effects of KA treatment on seizure activity and hippocampal cell death

We examined whether KA affects seizure severity after KA systemic injection. First, we investigated the properties of status epilepticus induced by KA injection with EEG recordings. A characteristic pattern of spontaneous seizures was observed in mice after KA injection (Fig. 1a). The frequency of these seizure spikes was dramatically increased in KA-treated mice compared with control mice. Consistent with EEG analysis, KA-injected mice showed a significant increase in seizure behaviours compared with control mice (data not shown). Furthermore, Cresyl violet-

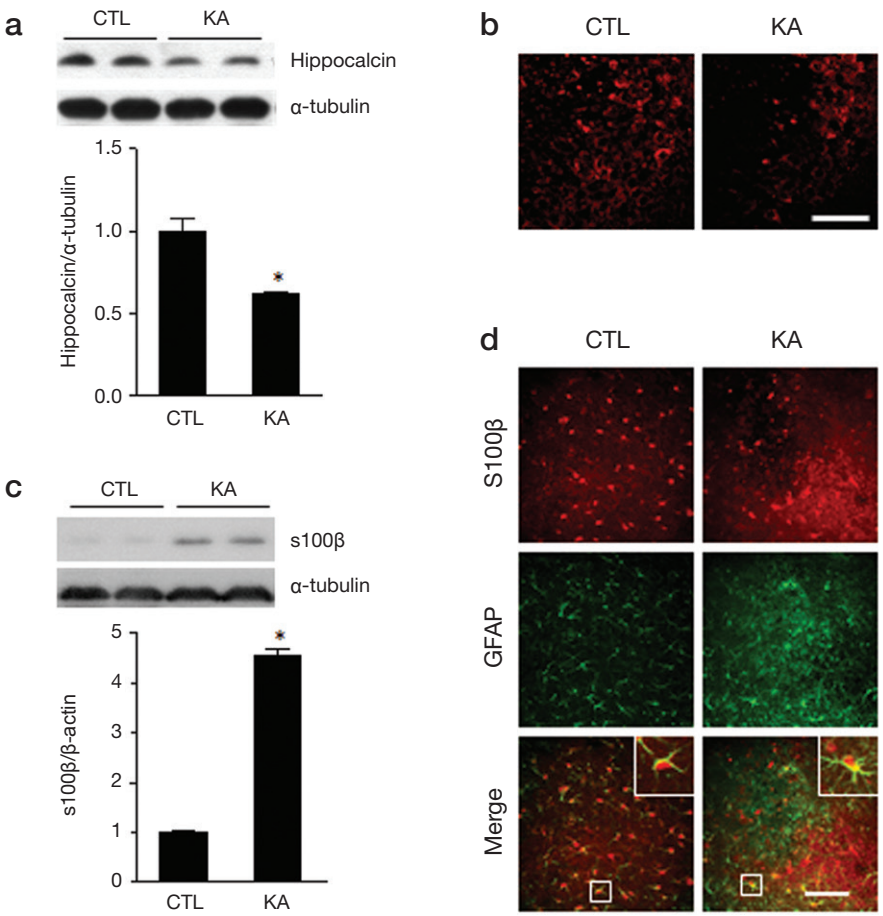

Fig. 2. Effects of KA treatment on Ca^{2+}-buffering proteins in the hippocampus. Mice were sacrificed at 24 h after KA injection. (a) Western blot analysis of hippocalcin. (b) Representative immunofluorescence images of hippocalcin in the CA3 region of the hippocampus. (c) Western blot analysis of S100β. (d) Representative immunofluorescence images of S100β (red) and GFAP (green) in the CA3 region of the hippocampus. Values were normalized to β-actin or α-tubulin. Data are shown as mean ± S.E.M. and the mean values were obtained from at least three independent experiment (n = 6 mice per group). *$p < 0.05$ versus control (CTL), Scale bar = 50 μm.

and TUNEL-stained sections of hippocampal CA3 regions were examined in KA injected mice and showed significantly increased numbers of the pyknotic nuclei typically found in apoptotic cells (Fig. 1b). The number of TUNEL-positive cells was increased in the hippocampus of KA-treated mice (22.6 ± 1.2, $p < 0.001$) compared with control mice. These results clearly indicate that KA-treated mice have susceptibility to experimentally-induced seizure activity and neuronal cell death.

2. Effects of KA treatment on Ca^{2+}-buffering protein levels in the mouse hippocampus

Next, we investigated expression of hippocalcin, a member of the neuronal Ca^{2+} sensor protein family, in the hippocampus of KA-treated mice. Hippocalcin plays an important role in Ca^{2+} extrusion from neurons and thereby protects them from Ca^{2+}-dependent excitotoxin damage in the hippocampus and its decreased expression increases seizure-induced neuronal cell death [20]. As shown in Fig. 2a, hippocalcin expression was significantly decreased by KA-treated mice compared to that of control mice. Immunohistochemical staining further revealed that KA injection decreased hippocalcin immunoreactivity in the hippocampus, but not in that of control mice (Fig. 2b). In contrast, we observed a dramatic increase in expression levels of the Ca^{2+}-sensing protein S100β in the KA-treated mice compared with control mice (Fig. 2c). S100β can increase intracellular free Ca^{2+} concentrations to regulate-neuron excitability [21,22], and immunohistochemical staining showed that KA injection can significantly increase the expression of S100β proteins in astrocytes (Fig. 2d) Collectively, KA-induced seizure generation may be due to increased Ca^{2+} influx in hippocampal neurons.

3. Effects of KA treatment on calpain-mediated proteolysis in the mouse hippocampus

To clarify the underlying molecular mechanisms linking KA-induced seizures, we investigated the calpain-mediated proteolysis pathway in KA-treated mice (Fig. 3). High concentrations of cytosolic Ca^{2+} constitutively activate calpain, which contributes to neuronal cell death by catalysing limited proteolysis of specific cellular protein such as spectrin breakdown product (SBDP), tau, and glycogen synthase kinase 3 beta (GSK3β) [23,24]. As shown in Fig. 3a, calpain 2 expression was remarkably increased in the

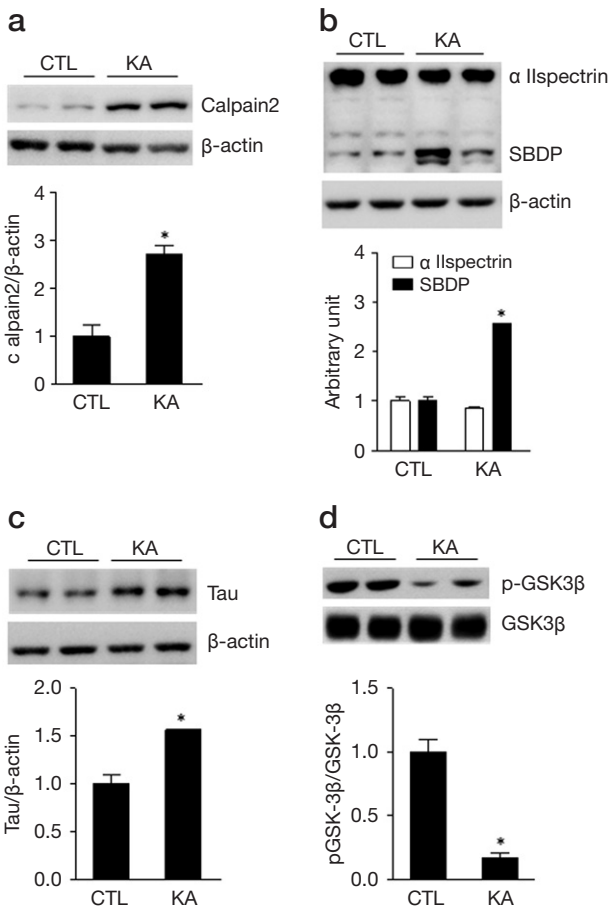

Fig. 3. Effects of KA treatment on calpain-mediated proteolysis in the hippocampus. Mice were sacrificed at 24 h after KA injection. Western blot analysis confirming the expression levels of (a) calpain 2, (b) full-length α-II spectrin and SBDP (fragments generated by calpain), (c) tau, and (d) GSK3β and [Ser9] GSK3β (p-GSK3B). The level of p-GSK3β was normalized to total GSK3β. Values were normalized to β-actin. Data are shown as mean ± S.E.M. and the mean values were obtained from at least three independent experiment ($n = 6$ mice per group). *$p < 0.05$ versus control (CTL).

hippocampus of KA-injected mice. Moreover, SBDP, a calpain-mediated breakdown product of α-II spectrin, did increase in the KA-treated mice compared to control mice (Fig. 3b). Interestingly, calpain-mediated proteolysis is tightly connected with tau expression and GSK3β phosphorylation in neuronal cell death [23]. Furthermore, KA-induced tau induction and GSK3β dephosphorylation (active form) was markedly increased or attenuated in the hippocampus, respectively (Fig. 3c and d). These results indicate that KA-induced excitotoxicity could be regulated by the calpain-mediated proteolysis pathway.

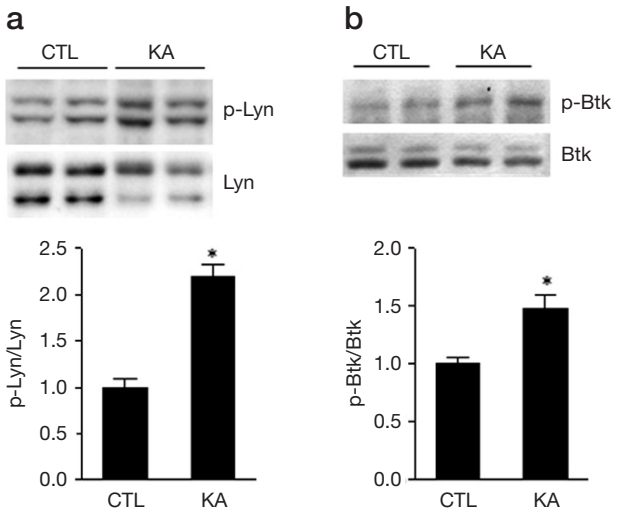

Fig. 4. Effects of KA treatment on phosphorylation of Lyn and Btk in the hippocampus. Western blot analysis confirming the expression levels of (a) Lyn and [Ser9] Lyn (p-Lyn), and (b) Btk and [Ser9] Btk (p-Btk). The levels of p-Lyn and p-Btk were normalized to total Lyn and total Btk, respectively. Data are shown as mean ± S.E.M. and the mean values were obtained from at least three independent experiment (n = 6 mice per group). *$p < 0.05$ versus control (CTL).

4. Effect of KA treatment on the phosphorylation of Lyn and Btk in the moue hippocampus

Previous reports showed that intracellular Ca^{2+} levels is regulated through the Lyn/Btk pathway [18]. In this study we first found that KA treatment increased phosphorylation of Lyn and Btk compared to control mice (Fig. 4a and b). These results suggest that the increase in Ca^{2+} influx caused by KA treatment is a critical step in the generation of seizures and neuronal hyperexcitability through Lyn/Btk pathway.

Discussion

This study provides the first evidence that KA-induced neuronal death is associated with the phosphorylation of Lyn/Btk. Previous studies have shown that Ca^{2+}-binding proteins and N-methyl-D-aspartate (NMDA) receptors play an important role in causing neuronal death followed by excitotoxicity [25-27]. However, the role of Lyn/Btk pathway in KA-induced neuronal cell death in the brain is largely unknown.

Seizure-induced neuronal cell death is associated with a complicated pathology, including Ca^{2+} overload, hyperexcitability, excitotoxicity, and neuroinflammation. Interestingly, we found a significant increase in seizure activity, Ca^{2+}-mediated excitotoxicity, and neuronal cell death in the mouse hippocampus after KA injection. These results suggest that the more complex of Ca^{2+}-mediated signalling pathway is pivotally involved in the pathology of KA-induced seizures, and that theirs inhibition may ameliorate Ca^{2+}-mediated excitotoxicity.

Glutamate is the major excitatory neurotransmitter in the brain, and seizure-induced neurodegeneration is caused by over-activation of glutamate receptors, which leads to excessive Ca^{2+} influx within neurons, resulting in neuronal damage [28]. Neuronal damage in meningitis may also be caused by excessive Ca^{2+} influx triggered by excitatory neurotransmitters [29]. The molecular mechanisms and contributors to excitotoxic neuronal cell death have been extensively investigated in various CNS disturbances, but the exact mechanisms and contributors are not fully understood. One aim of the present study was to elucidate the altered expression of Lyn/Btk in excitotoxic neuronal cell death. It has been reported that phosphorylated NMDA receptor subtype 2B (NR2B) can increase Ca^{2+} permeability through NMDA receptors [30]. NR2B has been identified as an indicator of intracellular Ca^{2+} influx that is activated by seizures [31].

In the present study, hippocalcin and S100β expression levels were measured as a surrogate neuronal or astrocytic calcium sensor because we did not directly measure Ca^{2+} levels. Hippocalcin plays an important role both in calcium extrusion from neurons and as a buffer for intracellular calcium levels, which in turn, protects against Ca^{2+}-dependent excitotoxicity in the KA-treated hippocampus. Our data are consistent with a previous study that observed reduced hippocalcin expression after the effects of KA injection were inhibited by ER stress inhibitors [8]. Previous studies have also shown the presence of pyknotic cells undergoing apoptosis in the hippocampus after KA injection in hippocalcin KO mice [20]. Glutamate exposure has been observed to increase intracellular Ca^{2+} levels and reduce hippocalcin expression in HT22 hippocampal cells [32]. Levels of hippocampal S100β, a calcium-binding protein predominantly found in astrocytes, are increased by seizures and associated with upregulation of GFAP protein expression [22]. In addition, S100β can increase intracellular free calcium concentrations and thereby reg-

ulate neuronal excitability. Chronic epilepsy causes overexpression of S100β by astrocytes under conditions of neuronal damage [33]. Moreover, another study observed that S100β KO mice kindled more rapidly and exhibited more severe seizures than control mice [34]. Thus, our observations of increased hippocalcin and S100β expression by KA treatment suggest that modulation of Ca^{2+} influx in the neurons and astrocytes in KA-treated mice may protect against Ca^{2+} overload-induced neuronal apoptosis.

Interestingly, the expression of hippocampal calpain 2 was significantly increased by KA treatment. Overstimulated ionotropic glutamate receptors, such as NMDA receptors, exert their neurotoxic effects in part by overactivation of calcium-dependent proteases, including calpain [35]. Moreover, calpains are calcium-dependent proteases known to be involved in neuronal cell death induced by excitotoxicity through the proteolysis of α-II spectrin into fragmentized products [24]. Calpains are also involved in the activation of GSK3β (de-phosphorylated form), and in tau phosphorylation [36]. Our findings indicate that seizure mice exhibit a significant increase in calpain-mediated neurotoxic signalling after KA injection.

Consistent with previous studies of Ca^{2+}-mediated neuronal excitotoxicity, we report the first observation of increased phosphorylation of Lyn and Btk in the hippocampus of KA-treated mice compared with control mice. According to previous reports, 4-1BB-activated signalling dramatically increases intracellular Ca^{2+} levels through phosphorylation of Src family kinases such as Lyn and Btk in lymphocytes [18,37]. In allergic reactions mediated by mast cells, both phosphorylation of Lyn and Btk are significantly decreased in 4-$1BB^{-/-}$ mast cells compared with wild type cells [18]. These findings suggest that the inhibition of activated calcium signalling by Lyn and Btk in KA-treated hippocampus could affect neuroprotection against KA-induced seizures.

In conclusion, recent reports and our results demonstrate that Ca^{2+}-mediated signalling is a crucial regulator of excitotoxicity-induced neuronal death in the brain. We conclude that Lyn/Btk pathway, as a modulator of calcium influx, plays an important role in neural activity, synaptic plasticity, and neuroimmune response. Protein kinase pathway is therefore attractive as a therapeutic target for the treatment of brain injuries and neurodegenerative disorders in which excitotoxic neuronal cell death and inflammation are involved.

REFERENCES

1. Simonian NA, Getz RL, Leveque JC, Konradi C, Coyle JT. Kainic acid induces apoptosis in neurons. Neuroscience. 1996; 75:1047-55.
2. Shin HJ, Lee JY, Son E, Lee DH, Kim HJ, Kang SS, et al. Curcumin attenuates the kainic acid-induced hippocampal cell death in the mice. Neurosci Lett. 2007; 416:49-54.
3. Lee JY, Jeon BT, Shin HJ, Lee DH, Han JY, Kim HJ, et al. Temporal expression of AMP-activated protein kinase activation during the kainic acid-induced hippocampal cell death. J Neural Transm. 2009; 116:33-40.
4. Ferkany JW, Zaczek R, Coyle JT. Kainic acid stimulates excitatory amino acid neurotransmitter release at presynaptic receptors. Nature. 1982; 298:757-9.
5. Jeong EA, Jeon BT, Shin HJ, Kim N, Lee DH, Kim HJ, et al. Ketogenic diet-induced peroxisome proliferator-activated receptor-gamma activation decreases neuroinflammation in the mouse hippocampus after kainic acid-induced seizures. Exp Neurol. 2011; 232:195-202.
6. Shin HJ, Jeon BT, Kim J, Jeong EA, Kim MJ, Lee DH, et al. Effect of the calcineurin inhibitor FK506 on K^+-Cl^- cotransporter 2 expression in the mouse hippocampus after kainic acid-induced status epilepticus. J Neural Transm. 2012; 119:669-77.
7. Wang GJ, Thayer SA. Sequestration of glutamate-induced Ca^{2+} loads by mitochondria in cultured rat hippocampal neurons. J Neurophysiol. 1996; 76:1611-21.
8. Kim JS, Heo RW, Kim H, Yi CO, Shin HJ, Han JW, et al. Salubrinal, ER stress inhibitor, attenuates kainic acid-induced hippocampal cell death. J Neural Transm. 2014; 121: 1233-43.
9. Noh HS, Kang SS, Kim DW, Kim YH, Park CH, Han JY, et al. Ketogenic diet increases calbindin-D28k in the hippocampi of male ICR mice with kainic acid seizures. Epilepsy Res. 2005; 65:153-9.
10. Sung JH, Kim MO, Koh PO. Ferulic acid attenuates the focal cerebral ischemic injury-induced decrease in parvalbumin expression. Neurosci Lett. 2012; 516:146-50.
11. Koh PO. Melatonin regulates the calcium-buffering proteins, parvalbumin and hippocalcin, in ischemic brain injury. J Pineal Res. 2012; 53:358-65.
12. Wang H, Yu SW, Koh DW, Lew J, Coombs C, Bowers W, et al. Apoptosis-inducing factor substitutes for caspase executioners in NMDA-triggered excitotoxic neuronal death. J Neurosci. 2004; 24:10963-73.
13. Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell. 1996; 86:147-57.
14. Siman R, Noszek JC, Kegerise C. Calpain I activation is

specifically related to excitatory amino acid induction of hippocampal damage. J Neurosci. 1989; 9:1579-90.
15. Croall DE, DeMartino GN. Calcium-activated neutral protease (calpain) system: structure, function, and regulation. Physiol Rev. 1991; 71:813-47.
16. Kurosaki T. Functional dissection of BCR signaling pathways. Curr Opin Immunol. 2000; 12:276-81.
17. Chung SC, Limnander A, Kurosaki T, Weiss A, Korenbrot JI. Coupling Ca^{2+} store release to Icrac channel activation in B lymphocytes requires the activity of Lyn and Syk kinases. J Cell Biol. 2007; 177:317-28.
18. Nishimoto H, Lee SW, Hong H, Potter KG, Maeda-Yamamoto M, Kinoshita T, et al. Costimulation of mast cells by 4-1BB, a member of the tumor necrosis factor receptor superfamily, with the high-affinity IgE receptor. Blood. 2005; 106:4241-8.
19. Shin HJ, Kim H, Heo RW, Kim HJ, Choi WS, Kwon HM, et al. Tonicity-responsive enhancer binding protein haplodeficiency attenuates seizure severity and NF-kappaB-mediated neuroinflammation in kainic acid-induced seizures. Cell Death Differ. 2014; 21:1095-106.
20. Masuo Y, Ogura A, Kobayashi M, Masaki T, Furuta Y, Ono T, et al. Hippocalcin protects hippocampal neurons against excitotoxin damage by enhancing calcium extrusion. Neuroscience. 2007; 145:495-504.
21. Barger SW, Van Eldik LJ. S100 beta stimulates calcium fluxes in glial and neuronal cells. J Biol Chem. 1992; 267: 9689-94.
22. Sakatani S, Seto-Ohshima A, Shinohara Y, Yamamoto Y, Yamamoto H, Itohara S, et al. Neural-activity-dependent release of S100B from astrocytes enhances kainate-induced gamma oscillations in vivo. J Neurosci. 2008; 28:10928-36.
23. Crespo-Biel N, Canudas AM, Camins A, Pallas M. Kainate induces AKT, ERK and cdk5/GSK3beta pathway deregulation, phosphorylates tau protein in mouse hippocampus. Neurochem Int. 2007; 50:435-42.
24. Liu J, Liu MC, Wang KK. Calpain in the CNS: from synaptic function to neurotoxicity. Sci Signal. 2008; 1:re1.
25. Brady RJ, Smith KL, Swann JW. Calcium modulation of the N-methyl-D-aspartate (NMDA) response and electrographic seizures in immature hippocampus. Neurosci Lett. 1991; 124:92-6.
26. Kamphuis W, Hendriksen H, Diegenbach PC, Lopes da Silva FH. N-methyl-D-aspartate and kainate receptor gene expression in hippocampal pyramidal and granular neurons in the kindling model of epileptogenesis. Neuroscience. 1995; 67:551-9.
27. Kim JE, Kwak SE, Kim DS, Won MH, Kwon OS, Choi SY, et al. Reduced calcium binding protein immunoreactivity induced by electroconvulsive shock indicates neuronal hyperactivity, not neuronal death or deactivation. Neuroscience. 2006; 137:317-26.
28. Olney JW. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science. 1969; 164:719-21.
29. Guerra-Romero L, Tureen JH, Fournier MA, Makrides V, Tauber MG. Amino acids in cerebrospinal and brain interstitial fluid in experimental pneumococcal meningitis. Pediatr Res. 1993; 33:510-3.
30. Viviani B, Bartesaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T, et al. Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. J Neurosci. 2003; 23:8692-700.
31. Hellier JL, White A, Williams PA, Dudek FE, Staley KJ. NMDA receptor-mediated long-term alterations in epileptiform activity in experimental chronic epilepsy. Neuropharmacology. 2009; 56:414-21.
32. Koh PO. Ferulic acid prevents cerebral ischemic injury-induced reduction of hippocalcin expression. Synapse. 2013; 67:390-8.
33. Yardan T, Erenler AK, Baydin A, Aydin K, Cokluk C. Usefulness of S100B protein in neurological disorders. J Pak Med Assoc. 2011; 61:276-81.
34. Dyck RH, Bogoch II, Marks A, Melvin NR, Teskey GC. Enhanced epileptogenesis in S100B knockout mice. Brain Res Mol Brain Res. 2002; 106:22-9.
35. Hossain MI, Roulston CL, Kamaruddin MA, Chu PW, Ng DC, Dusting GJ, et al. A truncated fragment of Src protein kinase generated by calpain-mediated cleavage is a mediator of neuronal death in excitotoxicity. J Biol Chem. 2013; 288:9696-709.
36. Feng Y, Xia Y, Yu G, Shu X, Ge H, Zeng K, et al. Cleavage of GSK-3beta by calpain counteracts the inhibitory effect of Ser9 phosphorylation on GSK-3beta activity induced by H_2O_2. J Neurochem. 2013; 126:234-42.
37. Nam KO, Shin SM, Lee HW. Cross-linking of 4-1BB upregulates IL-13 expression in CD8 (+) T lymphocytes. Cytokine. 2006; 33:87-94.

마우스 해마에서 카인산이 칼슘 관련 신경흥분독성에 미치는 영향

이정은

경상대학교 의학전문대학원 흉부외과학교실

간추림 : 카인산에 의한 신경세포 사멸은 세포 내 칼슘의 유입과 관련이 있다. 하지만 카인산에 의한 칼슘 관련 신경흥분독성과 신경세포 사멸에 있어 Lyn/Btk 경로가 관여한다는 것은 명확히 밝혀진 것이 없다. 따라서 본 연구에서는 카인산을 주사한 마우스 해마에서 발현 차이를 보이는 칼슘조절단백질들을 규명하고자 하였다. 마우스는 몸무게 당 카인산 20 mg을 복강주사하였으며 하루 뒤에 희생하였다. 전기뇌파검사를 통해 발작 활성도를 측정하였으며, 웨스턴블롯검사와 면역조직화학법을 이용하여 정상 마우스와 카인산 주사 마우스 해마조직의 단백질 발현 차이를 조사하였다. 카인산 주사 마우스는 정상 마우스에 비해 발작 활성도가 증가하였으며, 해마 부위에서 신경세포 사멸이 다수 관찰되었다. 카인산 투여는 칼슘결합단백질인 hippocalin 발현을 감소시켰으며 calpain 관련 단백질 분해는 증가되었다. 특히, Lyn과 Btk의 인산화 발현은 카인산에 의해 모두 증가되었다. 결론적으로 Lyn/Btk 신호전달 경로는 카인산 유도 발작에 의한 칼슘 관련 신경흥분독성의 중요한 조절자로 역할을 할 것으로 사료된다.

찾아보기 낱말 : 카인산, 칼슘, Lyn/Btk, 발작, 해마

교신저자 : 이정은(경상대학교 의학전문대학원 흉부외과학교실)
전자우편 : ilikerem@hanmail.net