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Short communication

Calpain-dependent cleavage of GABAergic proteins during epileptogenesis

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ABSTRACT

Epileptogenesis is the processes by which a normal brain transforms and becomes capable of generate spontaneous seizures. In acquired epilepsy, it is thought that epileptogenesis can be triggered by a brain injury but the understanding of the cellular or molecular changes unraveling is incomplete. In the CA1 region of hippocampus less GABAergic activity precede the appearance of spontaneous seizures and calpain overactivation has been detected after chemoconvulsant-induced status epilepticus (SE). Inhibition of calpain overactivation following SE ameliorates seizure burden, suggesting a role for calpain dysregulation in epileptogenesis. The current study analyzed if GABAergic proteins (*i.e.*, gephyrin, the vesicular GABA transporter and the potassium chloride co-transporter 2) undergo calpain-dependent cleavage during epileptogenesis. A time-dependent generation of break down products (BDPs) for these proteins was observed in the CA1 region of hippocampus after pilocarpine-induced SE. Generation of these BDPs was partially blocked by treatment with the calpain inhibitor MDL-28170. These findings suggest that calpain-dependent loss of GABAergic proteins might promote the erosion of inhibitory drive and contribute to hyperexcitability during epileptogenesis.

1. Introduction

The cellular and molecular events that transform the circuitry of a normal brain into hyperexcitable networks might result in the manifestation of overt spontaneous seizures as part of a process known as epileptogenesis (Fritschy, 2008; Pitkanen et al., 2015). Experimentation in animal models of epilepsy has revealed the existence of a transient decrease in GABAergic drive that coincides with appearance of epileptiform activity and spontaneous recurrent seizures within the hippocampus (El-Hassar et al., 2007). A loss of GABAergic drive and neuronal hyperexcitability appears to result from a loss of fully functional GABAergic proteins (Fritschy, 2008). Some of the proteins know to be down-regulated during epileptogenesis include the loss of $GABA_AR$ and its scaffolding protein, gephyrin (Gonzalez et al., 2013).

The calpains (*cal*cium-dependent proteases with pa*pain*-like activity) belong to a family of cysteine proteases activated by calcium (Campbell and Davies, 2012). A severe brain injury might result in a sustained increase in the levels of intracellular calcium that in turn promotes aberrant calpain activation (Saatman et al., 2010). Sustained calpain dysregulation contributes to acute and chronic neurodegeneration in many pathologic conditions (Vanderklish and Bahr, 2000; Saatman et al., 2010). As such, calpain dysregulation is observed after chemoconvulsant-induced *SE* and its pharmacological inhibition provides neuroprotection (Araujo et al., 2008; Wang et al., 2008) and ameliorates seizure burden (Lam et al., 2017).

Key calpain substrates located at GABAergic synapses include gephyrin, the vesicular GABA transporter (VGAT) and the potassium chloride co-transporter 2 (KCC2). Cleavage of gephyrin by calpain produces a fragment of ~45 kDa (Costa et al., 2016), cleavage of VGAT a fragment of ~35 kDa (Gomes et al., 2011) and cleavage of KCC2 a fragment of ~120 kDa (Zhou et al., 2012). The generation of these fragments has been observed both in "*in vitro*" experiments using purified calpain and following the induction of excitotoxic injuries (Gomes et al., 2011; Tyagarajan et al., 2011; Puskarjov et al., 2012). Gephyrin BDPs generated by calpain exert a dominant-negative effect and promote the disassembly of postsynaptic gephyrin clusters (Costa et al., 2016). Calpain-mediated cleavage of VGAT alters its permanence within nerve terminals impacting vesicle refilling and GABA release (Gomes et al., 2011). KCC2 cleavage weakens the chloride transmembrane gradient facilitating seizure onset (Wan et al., 2018).

In a previous study, we demonstrated that administration of MDL-28170, a calpain inhibitor, immediately after pilocarpine-induced SE partially prevents calpain overactivation and reduces total seizure burden (Lam et al., 2017). As a follow-up to those studies, this report describes the calpain-dependent cleavage of key GABAergic proteins that occurs during epileptogenesis. The results presented suggest that

Abbreviations: GABA, gamma-aminobutyric acid; $GABA_AR$, $GABA_A$ receptors; BDPs, break-down products; SE, status epilepticus; VGAT, vesicular GABA transporter; KCC2, potassium chloride co-transporter 2; CA1, Cornus Ammonis 1; PBS, phosphate buffered saline

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calpain inhibition after an epileptogenic injury might prevent the loss of proteins required for normal inhibitory neurotransmission.

2. Materials and methods

2.1. Neuronal cultures

Neurons were cultured from rat hippocampal tissue at postnatal day 0–2 (P0-P2), as described (Gonzalez, 2014). Briefly, hippocampal tissue was dissociated with papain by mechanical trituration and plated in MEM media supplemented with 10% fetal bovine serum (4 ml at 400,000 cells/ml). After one day in culture, cells were switched to Neurobasal-A media supplemented with B27 and inhibitors of glial proliferation. Cultures were maintained at 37 °C and 5% CO_2 and used after 14–15 days *in vitro* (DIV). To study calpain-dependent proteolysis, cells were incubated with glutamate plus glycine (100 μM each in HBSS) for 30 min and returned to conditioned culture media for up to six hours. To analyze calpain inhibition, cells were stimulated the presence of DMSO or MDL-28170 (100 μM). Cell lysates were prepared in RIPA buffer containing protease and phosphatase inhibitors. Protein concentration was determined using the Bio-Rad RC/DC reagent kit (Bio-Rad Laboratories, Hercules, CA, USA).

2.2. Pilocarpine model

Animal procedures were performed in accordance with Institutional Animal Care and Use Committee regulations and approved protocols by the University of Colorado Anschutz Medical Campus. Adult male Sprague Dawley rats (Charles River, Wilmington, MA) were housed in a temperature-controlled environment with food and water *ad libitum*. Status epilepticus (SE) was induced using a standard protocol (Gonzalez et al., 2013; Lam et al., 2017). Briefly, rats were injected with scopolamine (1 mg/kg) followed by pilocarpine (385 mg/kg). Diazepam (6 mg/kg; Hospira, Lake Forest, IL) was administered 1 h after SE to stop seizure progression. Control rats were handled similarly but treated with a subconvulsive dose of pilocarpine (1/10 of the full dose) and a reduced dose of diazepam (1/10 of the full dose). To determine the time-course of the cleavage of GABAergic proteins by calpain, in addition to control samples, tissue samples were collected at 1, 4 or 8 days post-SE (n = 6 per group). MDL-28170 is a cell permeable calpain inhibitor that inhibits both calpain-1 and calpain-2 (Markgraf et al., 1998; Thompson et al., 2010). The concentration and frequency of administration were chosen based on previous studies describing MDL-28170 delivery to the brain (Li et al., 1998; Markgraf et al., 1998; Araujo et al., 2008). MDL-28170 (50 mg/Kg, i.p. Bachem, Torrance, CA) was administered as previously described (Lam et al., 2017), a *low-dose treatment* (*two acute* injections applied at 1 and 5 h after *SE* onset and a final dose the following morning); and, a *high-dose treatment* (*four acute* doses at 1, 3, 5 and 9 h after *SE* onset and a final dose the following morning). For injection, a dilution of MDL-28170 was prepared at a concentration of 100 mg per ml requiring a volume of ~150 μl per injection. The vehicle group received DMSO instead of the drug. These treatment paradigms were previously used to evaluate the disease-modifying effect of calpain inhibition on seizure burden. The low-dose treatment resulted on a partial but significant reduction on seizure burden (Lam et al., 2017). To determine effects of the calpain inhibitor, tissue samples were collected from four groups of animals: control, vehicle treated and *low-dose* and *high-dose* treatments (n = 8 per group). To collect the tissue samples, the Cornus Ammonis 1 (CA1) was isolated from hippocampal slices (600 μm) prepared using a McIlwan tissue chopper (Gonzalez et al., 2013). Tissue lysates were prepared by brief sonication in RIPA buffer containing a mixture of protease and phosphatase inhibitors. Protein concentration was determined using the Bio-Rad RC/DC reagent kit (Bio-Rad Laboratories, Hercules, CA, USA).

2.3. Western blot

A polyclonal rabbit antibody (AB38, that only recognizes calpain-cleaved αII-spectrin fragments of ~150 kDa) produced and previously characterized (Roberts-Lewis et al., 1994) was generously provided by Dr. David R. Lynch (University of Pennsylvania, PA). A rabbit monoclonal antibody (clone EPR3017) to detect full-length and cleaved αII-spectrin (epitomics Cat. No. 2507-1, Abcam ID: ab75755) was obtained from Epitomics an Abcam company (Burlingame, CA). A mouse monoclonal antibody (clone 3B11) against the C6 domain of gephyrin (Cat. No. 147 111) and a guinea pig polyclonal antiserum for the N-terminus (a.a. 2-115) of VGAT (Cat. No. 131 004) were from Synaptic Systems (Gottingen, Germany). A rabbit polyclonal antibody against KCC2 N-terminus (Cat. No. SC-19419-R) was from Santa Cruz Biotechnology Inc. (Dallas, TX). An anti-actin antibody (Cat. No. A2066) was from Sigma (St. Louis, MO). Secondary antibodies, mouse anti-rabbit (GE Health Care, Piscataway, NJ), goat anti-mouse or donkey anti-guinea pig (Jackson Immunoresearch laboratories (West Grove, PA) were used. Samples were separated in SDS-polyacrylamide gels and transferred to nitrocellulose membranes. Blots were blocked for 1 h at room temperature and incubated overnight with primary antibodies. Secondary antibody was incubated for 1 h at room temperature. Immunoreactive bands were visualized using Super Signal West Dura chemiluminiscent substrate (Pierce, Rockford, IL, USA). Immunoreactive bands were quantified using Image J (NIH, Bethesda, MD, USA). Immunoreactivity was normalized to actin and compared to control values. Data is presented as the mean ± SEM.

2.4. Statistical analysis

Statistical analyses were performed using GraphPad InStat (GraphPad Software, Inc., San Diego, CA, USA). Differences between groups were determined by one-way analysis de variance (ANOVA) followed by Bonferroni *post hoc* test. For analyses, p values < 0.05 were considered significant.

3. Results

An *in vitro* characterization of calpain-dependent generation of BDPs for gephyrin, VGAT and KCC2 was carried out in cultured neurons exposed to excitotoxic conditions (glutamate plus glycine, 100 μM each, 30 min) known to promote calpain overactivation (Simpkins et al., 2003). This stimulation triggered a time-dependent generation of αII-spectrin BDPs detectable with AB38 and αII-spectrin antibodies (Fig. 1A). After confirmed calpain activation, cleavage of GABAergic proteins was analyzed. Time-dependent generation of BDPs for gephyrin, VGAT and KCC2 (of ~45, ~35 and ~120 kDa, respectively) was also detected (Fig. 1C). These GABAergic proteins showed differential susceptibility to cleavage, KCC2 appears to be most susceptible, followed by gephyrin and VGAT. To confirm that the BDPs were due to calpain activation, neurons were stimulated in the presence of MDL-28170 (100 μM). A reduction in αII-spectrin BDPs was detected (Fig. 1E, F). In addition, appearance of gephyrin, VGAT and KCC2 BDPs was also reduced, suggesting that the cleavage is calpain-dependent (Fig. 1G).

We recently reported an increase in αII-spectrin BDPs at 24 h after SE that is partially blocked by MDL-28170 treatment (Lam et al., 2017). To first investigate the *in vivo* cleavage of GABAergic proteins, western blots were carried out using CA1 samples collected at 1, 4 or 8 days post-*SE*. BDPs for gephyrin, VGAT and KCC2 (of ~45, ~35 and ~120 kDa, respectively) were detected within 24 h of SE (Fig. 2A). Like in the *in vitro* experiments, KCC2 appears to be most sensitive, followed by gephyrin and VGAT (Fig. 2B). Since MDL-28170 administration (50 mg/Kg i.p.) partially prevents calpain overactivation (Lam et al., 2017), the effects of MDL-28170 on the cleavage of GABAergic proteins was analyzed in tissue from animals treated with either a *low-* or *high-*

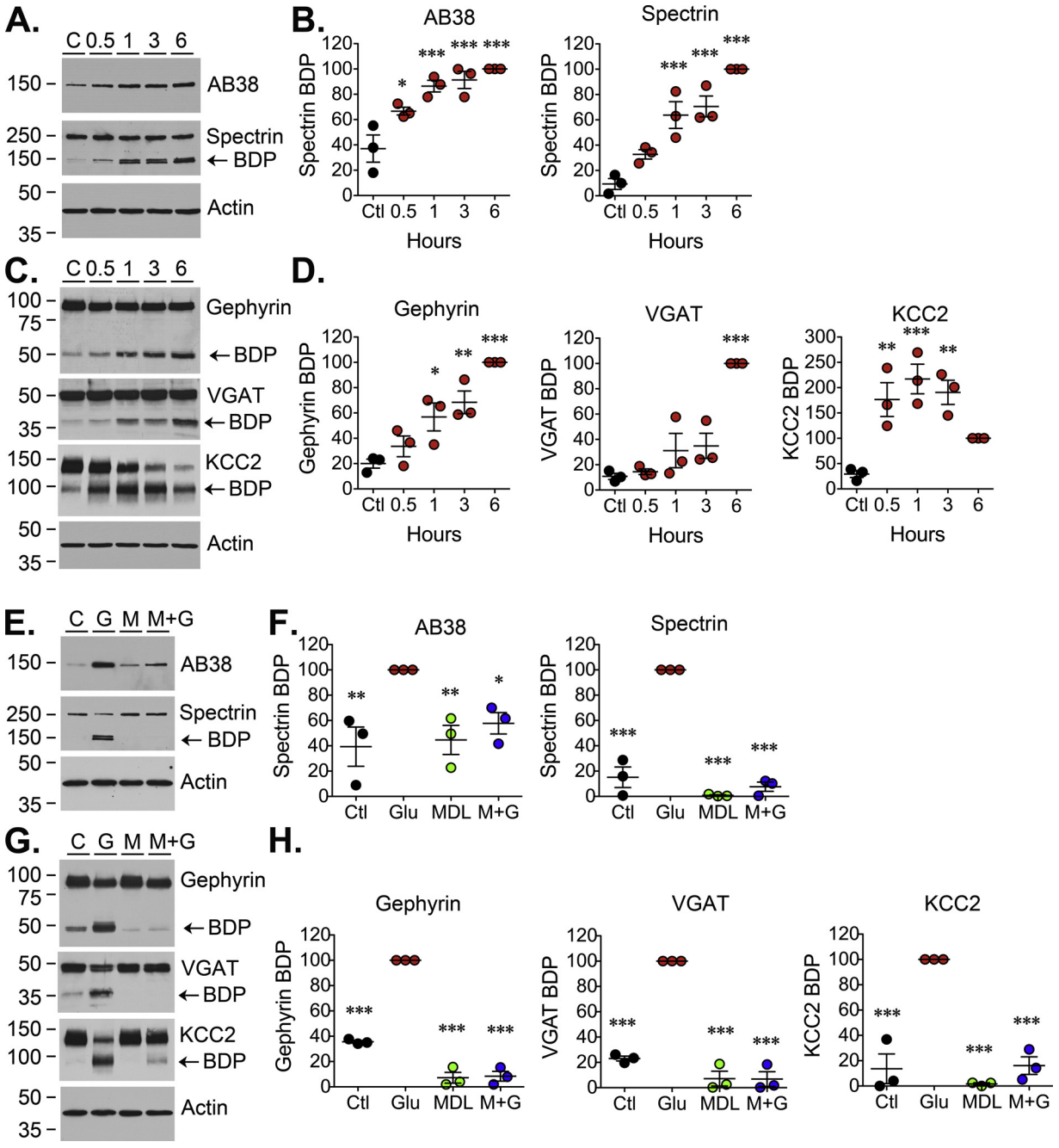

Fig. 1. ***Calpain-dependent cleavage of GABAergic proteins in cultured neurons.*** Hippocampal neurons were incubated with glutamate plus glycine (100 μM each) to promote calpain activation. Samples were collected at different time points and neuronal lysates were probed to assess calpain activation and cleavage of GABAergic proteins. (**A**) αII-spectrin BDPs were detected with either AB38 or anti-αII-spectrin antibody in control samples and in samples obtained at 0.5, 1, 3 or 6 h after glutamate incubation. (**B**) Immunoreactivity detected with either AB38 or anti-αII-spectrin antibody was normalized to actin signal. (**C**) BDPs for gephyrin, VGAT or KCC2 detected in control samples or in samples incubated with glutamate to activate calpain. (**D**) Immunoreactivity detected for gephyrin, VGAT or KCC2 BDPs was normalized to actin. Data is presented as the mean ± SEM of three independent experiments performed in three independent culture preparations. The signal detected was compared to control by ANOVA followed by Bonferroni *post hoc* test (*$p < 0.05$, **$p < 0.01$, or ***$p < 0.001$). Neurons were preincubated with MDL-28170 (100 μM) for 15 min before glutamate plus glycine application was carried out in the presence of the inhibitor. Cell lysates were prepared six hours after the beginning of glutamate exposure. (**E**) Representative blots for αII-spectrin BDPs detected with AB38 or anti-αII-spectrin antibodies. C or Ctl is for Control cultures, G or Glu is for cultures treated with glutamate, M or MDL is for cultures treated with MDL-28170 and M + G is for cultures treated with MDL-28170 plus glutamate. (**F**) Densitometry analysis of immunoreactivity for BDPs detected with AB38 or anti-αII-spectrin antibody normalized to actin. (**G**) Representative blots for the detection of BDPs for gephyrin, VGAT or KCC2 in cells incubated with glutamate in the presence or absence of MDL-28170. (**H**) Densitometric analysis of the immunoreactivity for gephyrin, VGAT or KCC2 BDPs normalized to actin. Data is presented as the mean ± SEM of three independent experiments performed in three independent culture preparations. The signal detected under the different conditions was compared to the signal detected in cells exposed to glutamate by ANOVA followed by Bonferroni *post hoc* test (*$p < 0.05$, **$p < 0.01$, or ***$p < 0.001$).

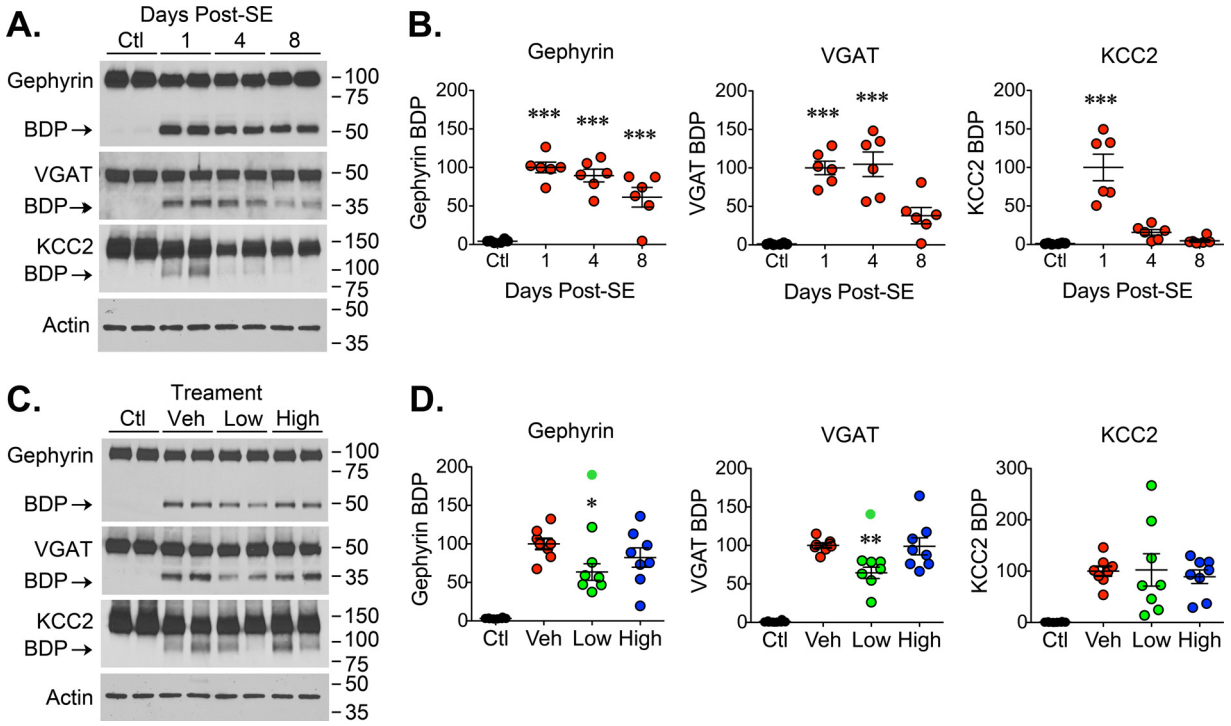

Fig. 2. ***Calpain-dependent cleavage of GABAergic proteins following SE.*** Tissue from the CA1 region of hippocampus was collected at different time points after SE induction (1, 4 and 8 days post SE) or from control animals (Ctl). Samples were analyzed by western blot to investigate the cleavage of GABAergic proteins by calpain. The appearance of BDPs was evaluated using antibodies for gephyrin, VGAT, KCC2 and actin. **(A)** Representative western blots showing BDPs of ~45, ~35 and ~120 kDa for gephyrin, VGAT and KCC2, respectively. These BDPs are characteristically released after calpain-dependent cleavage. **(B)** Quantitation of BDPs for gephyrin, VGAT or KCC2. Immunoreactivity for BDPs was normalized to actin and compared to the signal detected at one-day post SE. Data is presented as mean ± SEM of tissue samples obtained from six rats. The immunoreactivity of the BDPs detected in the different groups was compared by ANOVA followed by Bonferroni *post hoc* test (***$p < 0.001$ represents a significant difference). The effects calpain inhibition on the generation of BDPs was also analyzed in samples from CA1. Samples were obtained from control rats (Ctl) or from rats subject to SE and treated with either vehicle (Veh), or MDL-28170 at a *low*- or *high*-dose. **(C)** Representative western blots showing detection of BDPs for gephyrin, VGAT, KCC2 and actin. **(D)** Quantitation of BDPs detected for gephyrin, VGAT or KCC2. To quantitate appearance of BDPs after MDL-28170 treatment, BDP immunoreactivity was normalized to actin and compared to the signal detected in vehicle group (Veh). Data presented as the mean ± SEM of tissue samples obtained from seven (gephyrin and VGAT) or eight rats (KCC2). For gephyrin and VGAT, the value for one of the samples was excluded from the calculation (circles without border) because they were beyond two standard deviations of the mean and above any value obtained in vehicle treated samples. BDPs immunoreactivity in the different groups was compared by ANOVA followed by Bonferroni *post hoc* test (*$p < 0.05$ or **$p < 0.01$ represent a significant difference).

dose of MDL-28179 (Lam et al., 2017). In animals treated with *low*-dose MDL-28170, a significant reduction in BDPs for gephyrin and VGAT was observed (Fig. 2C, D). For KCC2, BDPs production was too variable and not significant (Fig. 2D). In addition, no significant effect was observed in tissue from animals treated with *high*-dose MDL-28170 (Fig. 2D). These observations are in agreement with our previous report where *low*-dose MDL-28170 was most effective in reducing calpain activation and total seizure burden (Lam et al., 2017).

4. Discussion

Calpain dysregulation has been observed after chemoconvulsant-induced *SE* and its pharmacological inhibition provides neuroprotection (Araujo et al., 2008) and ameliorates seizure burden (Lam et al., 2017). Loss of GABAergic proteins after chemoconvulsant-induced *SE* has been previously documented (Pathak et al., 2007; Barmashenko et al., 2011; Gonzalez et al., 2013) but its specific regulation remains poorly characterized. This study documents the cleavage of gephyrin, VGAT and KCC2 during the epileptogenic period that follows pilocarpine-induced SE. Administration of the calpain inhibitor MDL-28170 immediately after SE partially prevented cleavage of gephyrin and VGAT but did not protect KCC2. These observations suggest that calpain overactivation might compromise the integrity of GABAergic proteins promoting the erosion of inhibitory neurotransmission.

Gephyrin cleavage by calpain results in a loss of full-length gephyrin and concomitant accumulation of BDPs. Gephyrin BDPs might act as dominant negative entities that promote disassembly of the gephyrin lattice and the loss of anchoring sites for receptors at the synapse (Costa et al., 2016). A previous study demonstrated a time-dependent reduction in plasma membrane $GABA_AR$ that mimics the trend of calpain cleavage described here (Gonzalez et al., 2013). In mice subject to MCAO, VGAT is cleaved at both the ischemic core and penumbra regions altering its targeting and synaptic distribution (Gomes et al., 2011). VGAT is required to load GABA into synaptic vesicles and its cleavage might affect GABA release. Impairment in GABA functions produce seizures and its enhancement results in anticonvulsant effects (Fritschy, 2008). A positive shift in E_{GABA} is associated with KCC2 loss of expression (Pathak et al., 2007; Barmashenko et al., 2011). During epileptogenesis there is profound reduction in KCC2 cell surface levels within the CA1 region that is accompanied by a loss of total KCC2 (Gonzalez, 2016).

Under the experimental conditions used here, it appears to be a differential time-dependency for the cleavage of these proteins suggesting a differential susceptibility to calpain. KCC2 appears to be the most sensitive, followed by gephyrin and then VGAT. The specific reasons for this pattern of degradation are unknown but one could speculate that a steric effect governing the accessibility of a protein could be a factor. Another factor could be the amino acid sequences that serve to target proteins for calpain-mediated degradation (*i.e.* PEST sequences) present in these proteins (Gomes et al., 2011; Tyagarajan

et al., 2011; Puskarjov et al., 2012). However, specific role of these and another factors remain to be fully investigated.

Stability of GABAergic synapses is essential to maintain inhibitory drive and normal brain circuit function. Sensitivity of multiple GABAergic proteins to calpain cleavage suggests a more general role of calpain on the regulation of GABAergic synapses (Puskarjov et al., 2012). The consequences of this regulation are starting to emerge and mounting evidence supports that calpain overactivation contributes to seizure development affecting seizure onset and seizure burden (Lam et al., 2017; Wan et al., 2018). Further characterization of the role of calpain activation on GABAergic synapses is needed to determine if calpain is a suitable target to develop novel anti-epileptogenic drugs. Current clinical protocols do not consider epileptogenesis as a treatment indication and no therapies are available to treat individuals at risk of epileptogenesis (Pitkanen et al., 2015).

Declaration of Competing Interest

The author declares no conflict of interest.

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References

Araujo, I.M., Gil, J.M., Carreira, B.P., Mohapel, P., Petersen, A., Pinheiro, P.S., Soulet, D., Bahr, B.A., Brundin, P., Carvalho, C.M., 2008. Calpain activation is involved in early caspase-independent neurodegeneration in the hippocampus following status epilepticus. J. Neurochem. 105, 666–676.

Barmashenko, G., Hefft, S., Aertsen, A., Kirschstein, T., Kohling, R., 2011. Positive shifts of the GABAA receptor reversal potential due to altered chloride homeostasis is widespread after status epilepticus. Epilepsia 52, 1570–1578.

Campbell, R.L., Davies, P.L., 2012. Structure-function relationships in calpains. Biochem. J. 447, 335–351.

Costa, J.T., Mele, M., Baptista, M.S., Gomes, J.R., Ruscher, K., Nobre, R.J., de Almeida, L.P., Wieloch, T., Duarte, C.B., 2016. Gephyrin cleavage in in vitro brain ischemia decreases GABAA receptor clustering and contributes to neuronal death. Mol. Neurobiol. 53, 3513–3527.

El-Hassar, L., Esclapez, M., Bernard, C., 2007. Hyperexcitability of the CA1 hippocampal region during epileptogenesis. Epilepsia 48 (Suppl. 5), 131–139.

Fritschy, J.M., 2008. Epilepsy, E/I balance and GABA(A) receptor plasticity. Front. Mol. Neurosci. 1, 5.

Gomes, J.R., Lobo, A.C., Melo, C.V., Inacio, A.R., Takano, J., Iwata, N., Saido, T.C., de Almeida, L.P., Wieloch, T., Duarte, C.B., 2011. Cleavage of the vesicular GABA transporter under excitotoxic conditions is followed by accumulation of the truncated transporter in nonsynaptic sites. J. Neurosci. 31, 4622–4635.

Gonzalez, M.I., 2014. Brain-derived neurotrophic factor promotes gephyrin protein expression and GABAA receptor clustering in immature cultured hippocampal cells. Neurochem. Int. 72, 14–21.

Gonzalez, M.I., 2016. Regulation of the cell surface expression of chloride transporters during epileptogenesis. Neurosci. Lett. 628, 213–218.

Gonzalez, M.I., Cruz Del Angel, Y., Brooks-Kayal, A., 2013. Down-regulation of gephyrin and GABAA receptor subunits during epileptogenesis in the CA1 region of hippocampus. Epilepsia 54, 616–624.

Lam, P.M., Carlsen, J., Gonzalez, M.I., 2017. A calpain inhibitor ameliorates seizure burden in an experimental model of temporal lobe epilepsy. Neurobiol. Dis. 102, 1–10.

Li, P.A., Howlett, W., He, Q.P., Miyashita, H., Siddiqui, M., Shuaib, A., 1998. Postischemic treatment with calpain inhibitor MDL 28170 ameliorates brain damage in a gerbil model of global ischemia. Neurosci. Lett. 247, 17–20.

Markgraf, C.G., Velayo, N.L., Johnson, M.P., McCarty, D.R., Medhi, S., Koehl, J.R., Chmielewski, P.A., Linnik, M.D., 1998. Six-hour window of opportunity for calpain inhibition in focal cerebral ischemia in rats. Stroke 29, 152–158.

Pathak, H.R., Weissinger, F., Terunuma, M., Carlson, G.C., Hsu, F.C., Moss, S.J., Coulter, D.A., 2007. Disrupted dentate granule cell chloride regulation enhances synaptic excitability during development of temporal lobe epilepsy. J. Neurosci. 27, 14012–14022.

Pitkanen, A., Lukasiuk, K., Dudek, F.E., Staley, K.J., 2015. Epileptogenesis. Cold Spring Harb. Perspect. Med. 5.

Puskarjov, M., Ahmad, F., Kaila, K., Blaesse, P., 2012. Activity-dependent cleavage of the K-Cl cotransporter KCC2 mediated by calcium-activated protease calpain. J. Neurosci. 32, 11356–11364.

Roberts-Lewis, J.M., Savage, M.J., Marcy, V.R., Pinsker, L.R., Siman, R., 1994. Immunolocalization of calpain I-mediated spectrin degradation to vulnerable neurons in the ischemic gerbil brain. J. Neurosci. 14, 3934–3944.

Saatman, K.E., Creed, J., Raghupathi, R., 2010. Calpain as a therapeutic target in traumatic brain injury. Neurotherapeutics 7, 31–42.

Simpkins, K.L., Guttmann, R.P., Dong, Y., Chen, Z., Sokol, S., Neumar, R.W., Lynch, D.R., 2003. Selective activation induced cleavage of the NR2B subunit by calpain. J. Neurosci. 23, 11322–11331.

Thompson, S.N., Carrico, K.M., Mustafa, A.G., Bains, M., Hall, E.D., 2010. A pharmacological analysis of the neuroprotective efficacy of the brain- and cell-permeable calpain inhibitor MDL-28170 in the mouse controlled cortical impact traumatic brain injury model. J. Neurotrauma 27, 2233–2243.

Tyagarajan, S.K., Ghosh, H., Yevenes, G.E., Nikonenko, I., Ebeling, C., Schwerdel, C., Sidler, C., Zeilhofer, H.U., Gerrits, B., Muller, D., Fritschy, J.M., 2011. Regulation of GABAergic synapse formation and plasticity by GSK3beta-dependent phosphorylation of gephyrin. Proc. Natl. Acad. Sci. U. S. A. 108, 379–384.

Vanderklish, P.W., Bahr, B.A., 2000. The pathogenic activation of calpain: a marker and mediator of cellular toxicity and disease states. Int. J. Exp. Pathol. 81, 323–339.

Wan, L., Ren, L., Chen, L., Wang, G., Liu, X., Wang, B.H., Wang, Y., 2018. M-calpain activation facilitates seizure induced KCC2 down regulation. Front. Mol. Neurosci. 11, 287.

Wang, S., Shan, P., Song, Z., Dai, T., Wang, R., Chi, Z., 2008. Mu-calpain mediates hippocampal neuron death in rats after lithium-pilocarpine-induced status epilepticus. Brain Res. Bull. 76, 90–96.

Zhou, H.Y., Chen, S.R., Byun, H.S., Chen, H., Li, L., Han, H.D., Lopez-Berestein, G., Sood, A.K., Pan, H.L., 2012. N-methyl-D-aspartate receptor- and calpain-mediated proteolytic cleavage of K+-Cl- cotransporter-2 impairs spinal chloride homeostasis in neuropathic pain. J. Biol. Chem. 287, 33853–33864.