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Increased metalloproteinase activity in the hippocampus following status epilepticus

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ABSTRACT

Increased neuronal plasticity and neuronal cell loss has been implicated in the development of epilepsy following injury. Parvalbumin fast spiking inhibitory interneurons have a robust extracellular matrix coating their cell bodies and the proximal dendrites called the perineuronal net (PNN). The role of the PNN is not clear but it has been implicated in closing of the critical period, altering seizure thresholds and providing neuronal protection from oxidative stress. The PNN is susceptible to degradation following a prolonged seizure and there is an increase in proteolytic-fragments of the PNN enriched proteoglycan aggrecan (Dzwonek et al., 2004). Here we demonstrate an increase in matrix metalloproteinase (MMP) activity in the hippocampus following status epilepticus (SE). We further assessed MMP3 and 13, two of 24 identified MMPs, both MMP3 and 13 mRNA increase in the hippocampus after SE and MMP13 activity increases by functional assay as well as it co-localizes with PNN in rat brain. In contrast, two of the brain expressed ADAMTS (A Disintegrin And Metalloproteinase with ThromboSpondin motifs) also implicated in aggrecan degradation, did not consistently increase following SE though ADAMTS4 is highly expressed in glia and ADAMTS5 in neuronal cell bodies and their processes. The increase in MMP activity following SE suggests that in the future studies, MMP inhibitors are candidates for blocking PNN degradation and assessing the role of the PNN loss in epileptogenesis and cellular function.

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1. Introduction

Epilepsy prevention in high-risk patients has been a challenging problem. Drugs that effectively treat spontaneous seizures have been ineffective in preventing epilepsy. One reason may be that most anti-epileptic drugs dampen excitation or increase inhibition without focusing on the molecular and cellular changes that contribute to the development of epilepsy. Previously we published in a rodent model of epilepsy that perineuronal net (PNN) integrity is lost as animals develop epilepsy and remains diminished in animals with chronic epilepsy (McRae et al., 2012). The loss of the PNN may apply to other brain disorders such as stroke and schizophrenia (Karetko-Sysa et al., 2011; Mauney et al., 2013). The PNN is a multimeric proteoglycan complex that surrounds synapses on the cell body and proximal dendrites and is especially abundant around fast spiking-parvalbumin (FS-PV) interneurons.

The main components of the PNN are hyaluronic acid (HA), hyaluronan synthases (HASs), chondroitin sulfate proteoglycans – primarily lecticans, HA and proteoglycan link protein (HAPLNs), and tenascin-R (Galtrey et al., 2008; Carulli et al., 2010; Kwok et al., 2010; Giamanco and Matthews, 2012). Aggrecan is a PNN specific lectican, having several protein domains for cross linking to tenascin and HA and long stretches of chondroitin sulfate side chains. Hyaluronic acid is synthesized and remains attached to the plasma membrane by HASs (McRae and Porter, 2012). The PNN appears during the 2nd week of postnatal development and its components continue to increase throughout adulthood (McRae et al., 2010). The PNN has multiple functions, including neurotransmitter receptor synaptic stabilization, closing of the ocular dominance sensitive period, and neuroprotection (Cabungcal et al., 2013; Maroto et al., 2013). Proteoglycan degradation has been proposed as a therapeutic strategy to promote neuronal reorganization

Abbreviations: ADAMTS, A disintegrin and metalloproteinase with thrombospondin motifs; FS-PV, Fast spiking-parvalbumin neurons; GFAP, Glial fibrillary acidic protein; IP, Intraperitoneal; MAP2, Microtubule- associated protein 2; MMP, Matrix metalloproteinase; PNN, Perineuronal net; RT-PCR, Real time- polymerase chain reaction; SE, Status epilepticus.

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and recovery following trauma but also might contribute to the development of epilepsy (Kwok et al., 2008). Future studies will be needed to determine if disruption of the PNN contributes to epileptogenesis, memory problems and the changes in the electrophysiology of the FS-PV interneurons that contributes to seizure activity (Zhang and Buckmaster, 2009; Shiri et al., 2015; Toyoda et al., 2015).

Matrix metalloproteinases (MMPs), cleave extracellular proteins including aggrecan, are found throughout the CNS and are necessary for long-term potentiation and regulation of postsynaptic density morphology (Dzwonek et al., 2004). Recently, an increase in the MMP neo-epitopes of aggrecan following status epilepticus (SE) was demonstrated (Rankin-Gee et al., 2015). Only a few of the more than 24 MMPs have been studied in epilepsy, though not all are expressed in the brain. MMP9 and MMP13 are two MMPs found to increase following brain injury, although there are no prior studies exploring their role in degrading PNNs or aggrecan proteolysis in the brain (Nagel et al., 2005). In seizures, MMP9 mRNA and protein levels increase, accompanied by an increase in enzymatic activity (Konopacki et al., 2007; Mizoguchi et al., 2011; Hoehna et al., 2012). Furthermore, pentylenetetrazole (PTZ) kindling is inhibited in MMP9 knockout mice and kindling is increased in MMP9 overexpressing transgenic mice (Wilczynski et al., 2008). Following focal cerebral ischemia, there is up regulation and colocalization of MMP13 with aggrecan, suggesting PNN degradation may occur in response to a variety of brain injuries (Nagel et al., 2005). Another protease family known to cleave aggrecan but less studied in the CNS is ADAMTS (A Disintegrin And Metalloproteinase with ThromboSpondin motifs) proteases known as aggrecanases (Stanton et al., 2011; Gottschall and Howell, 2015). ADAMTS1, 4, 5 and 9, are expressed in the brain and are also candidates for aggrecan degradation following SE.

Here we focus on MMP13 and MMP3, and ADAMTS4 and 5 since they are expressed in the brain and known to specifically cleave aggrecan in cartilage (Fosang et al., 1991; Flannery et al., 1992; Fosang et al., 1996; Dzwonek et al., 2004; Cross et al., 2006; Held-Feindt et al., 2006). The role of the PNN in epileptogenesis or its ability to alter FS-PV cell function is unknown. Identification of proteases involved in degradation of the PNN and extracellular matrix after a brain insult will allow for targeted therapies for prevention of epilepsy in high-risk patients.

2. Material and methods

2.1. Animals

The Institutional Animal Care and Use Committees at the Children's Hospital of Philadelphia and Stanford University approved all the procedures. Male Sprague–Dawley (SD) rats approximately 250 gm in weight, from Charles River (Wilmington, MA, U.S.A.), were randomly assigned into either the control group or the status epilepticus (SE) group. Rats were single- housed in temperature and humidity controlled housing with a 12 h light- 12 h dark cycle and had *ad libitum* access to food and water.

2.2. Seizure induction

Status epilepticus was induced in the experimental group using the pilocarpine chemoconvulsant rodent model of epilepsy following same protocol as described in Dubey and Porter (2016). Briefly, male SD rats were injected with 1 mg/kg methyl-scopolamine intraperitoneally (IP) (Sigma-Aldrich Corp., MO) followed by IP injection of 385 mg/kg body weight of pilocarpine hydrochloride (Sigma-Aldrich Corp., MO). Control rats received sub-convulsive dose of 38.5 mg/kg body weight of pilocarpine. Rats were monitored for behavioral seizures and SE induction onset was measured from the start of the first stage five seizure on Racine scale (Racine, 1972). The seizure group received an IP injection of diazepam (6 mg/kg) (Hospira Inc., IL) 1 h after the onset of Racine stage five seizure, while the control group received the same dose 1 h after the low-dose pilocarpine injection. The seizure group received an additional 1/2 dose of diazepam (3 mg/kg) 2 h after the initial diazepam injection if the animal had ongoing seizure activity.

2.3. MMP activity

Status epilepticus was induced in male SD rats as described above. Hippocampi were quickly dissected from SE-induced and control rats euthanized at different time points after SE induction and frozen in dry ice. Hippocampal lysates were prepared using reagents provided in the SensoLyte® Plus 520 MMP13 Assay Kit (Cat # AS-72019) and SensoLyte® 520 Generic MMP Assay Kit (Catalogue # 71158) (Anospec, Fremont, CA) and assay for the MMP13 activity and total MMP activity in SE and control samples was performed following manufacturer's instructions. The fluorescent intensity of the samples was measured at Excitation/Emission wavelength = 490 nm/520 nm. All values were normalized to the values from control animals and plotted. Statistical analysis was performed using an analysis of variance (ANOVA) and post hoc Bonferroni's multiple comparisons test comparing seizure and control animals using Prism 5.0 software.

2.4. Real time-PCR (RT-PCR)

Real Time-PCR was performed as described in McRae et al. (2012). Briefly, whole hippocampal tissue samples were homogenized with a sonicator on ice. RNA was extracted from the tissue using the *mir*Vana Isolation Kit (Thermo Fisher Scientific, Waltham, MA). RNA concentrations were measured with a spectrophotometer (NanoDrop ND1000, Thermoscientific, Wilmington, DE). Five micrograms of purified RNA in 8 μls were reverse transcribed with the SuperScript II reverse transcription kit using random hexamer primers (Life Technologies, NY). The cDNA concentrations were quantified and diluted to 500 ng/2 μl sample that underwent RT-PCR in a 384-well plate. Each master mix was prepared using the Taqman Universal Master Mix (Life Technologies, NY) and a probe for MMP3 (RN00591740_m1), MMP13 (Rn01448194_m1), ADAMTS4 (AIRR82F: GTCCCCCTGCAGTGCCCGATTCAT-CACTGACTTCCTGGACAATGGCTATGGACACTGC-CTCTTAGACAAACCAGAGGCTCCCCTGCATCTGCCAGTGACT), or ADAMTS5 (Rn01458488_m1) (Thermo Fisher Scientific, Waltham, MA). Each sample was assayed in triplicate to minimize error and matched to a standard curve of rat cortex cDNA. The RT-PCR assay was executed by an SDS 7900HT thermocycler (Applied Biosystems by Thermo Fisher Scientific, Waltham, MA), comprised of a 2 min cycle at 50 °C, followed by a 10 min cycle at 95 °C, and 40 cycles of 15 s at 95 °C and 60 s at 60 °C. Data was expressed as a percent change relative to control values in the same run. Statistical analysis was performed using an analysis of variance (ANOVA) and post hoc Bonferroni's multiple comparisons test comparing seizure and control animals using Prism 5.0 software.

2.5. Western blotting

Fresh or frozen, whole hippocampal tissue samples were homogenized on ice with a sonicator. Protein concentrations were analyzed using the Bio-Rad Protein Assay (Bio-Rad, Hercules, CA) using a spectrophotometer. Equal amount of protein was added to 4 × NuPage LDS Sample Buffer (Life Technologies, NY) and 10 × NuPage Sample Reducing Agent to bring samples to a concentration of 1 μg/μl. This mixture was placed on a dry bath at 72–80 °C

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