EPILEPSY CURRENTS

Current Literature

In Basic Science

"Please Release Me, Let Me Go"—Changes in Presynaptic Release Following Status Epilepticus

Altered Neurotransmitter Release, Vesicle Recycling and Presynaptic Structure in the Pilocarpine Model of Temporal Lobe Epilepsy.

Upreti C, Otero R, Partida C, Skinner F, Thakker R, Pacheco LF, Zhou ZY, Maglakelidze G, Velíšková J, Velíšek L, Romanovicz D, Jones T, Stanton PK, Garrido-Sanabria ER. *Brain* 2012;135(pt 3):869–885.

In searching for persistent seizure-induced alterations in brain function that might be causally related to epilepsy, presynaptic transmitter release has relatively been neglected. To measure directly the long-term effects of pilocarpine-induced status epilepticus on vesicular release and recycling in hippocampal mossy fibre presynaptic boutons, we used (i) two-photon imaging of FM1-43 vesicular release in rat hippocampal slices; and (ii) transgenic mice expressing the genetically encoded pH-sensitive fluorescent reporter synaptopHluorin preferentially at glutamatergic synapses. In this study we found that, 1-2 months after pilocarpine-induced status epilepticus, there were significant increases in mossy fibre bouton size, faster rates of action potential-driven vesicular release and endocytosis. We also analysed the ultrastructure of rat mossy fibre boutons using transmission electron microscopy. Pilocarpine-induced status epilepticus led to a significant increase in the number of release sites, active zone length, postsynaptic density area and number of vesicles in the readily releasable and recycling pools, all correlated with increased release probability. Our data show that presynaptic release machinery is persistently altered in structure and function by status epilepticus, which could contribute to the development of the chronic epileptic state and may represent a potential new target for antiepileptic therapies.

Commentary

Investigations of neuronal changes following status epilepticus and mechanisms of epileptogenesis have typically focused on postsynaptic modifications involving neurotransmitter receptors and ion channels. There has been a relative lack of attention to presynaptic changes, despite the fact that presynaptic release of transmitters drives synaptic transmission. In addition, several anticonvulsants exert their action on the presynaptic side of the synapse. Possible presynaptic structural or physiological changes that could enhance excitability and increase the propensity to seizures include a greater number or more easily released synaptic vesicles containing excitatory neurotransmitter, faster release kinetics, more membrane area devoted to vesicle docking (active sites), and faster recycling of transmitter back into the presynaptic terminal to form a readily releasable pool. In one of the few previous studies evaluating presynaptic release changes in an epilepsy model, Goussakov and colleagues demonstrated enhanced glutamate release from the readily releasable vesicle pool in mossy fibers of rats that had undergone kainate-induced status epilepticus (1). Engelbert Humperdink, a legendary crooner, appreciated the importance of "release." In his signature song, Humperdink urged his lover to "please release me, let me go…and let me love again." In that spirit, the present authors investigated the changes in structure and function of the presynaptic terminals of mossy fiber boutons following pilocarpine-induced status epilepticus.

In the hippocampus, status epilepticus–induced cell death, particularly in the dentate hilus, is associated with sprouting of axonal terminals from dentate granule cells, which ordinarily innervate hilar interneurons and CA3 pyramidal neurons, into the dentate inner molecular layer. This anomalous innervation pattern, known as mossy fiber sprouting, presumably leads to enhanced circuit excitability and predisposes to seizure generation. Mossy fiber sprouting forms the morphologic basis of well-studied models of temporal lobe epilepsy (2). The aim of the present article is to examine changes in the presynaptic machinery of mossy fibers, to see whether any long-term physiological or ultrastructural changes occur following status epilepticus. In these experiments, P30-35 rats or mice were allowed to undergo pilocarpine-induced status epilepticus for defined durations (rats, 3 hours; mice, 1 hour) before terminating the status with diazepam.

It is challenging to perform single-cell electrophysiology on cells of the hippocampus after status epilepticus because of the extensive cell death in the region of interest. Instead, to approach the question, Upreti and colleagues employed two methods to visualize presynaptic release. First, they utilized two-photon laser scanning microscopy to directly visualize

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transmitter vesicles. A fluorescent dye, FM1-43, selectively labels transmitter vesicles of the readily releasable pool; the stimulus-evoked rate at which fluorescence dissipates (destaining kinetics) was determined in Sprague-Dawley rats 1 to 2 months after pilocarpine-induced status epilepticus compared with age-matched, non-status control rats. There was a marked increase in FM1-43 release in the status animals compared with controls. The effect was dependent upon neural activity, since spontaneous, action potential–independent vesicle release was not associated with differences in fluorescence kinetics.

A second method independently assessed vesicle release utilizing two-photon laser scanning microscopy in transgenic mice that selectively express the pH-sensitive vesicle fusion protein synaptopHluorin (SpH). SpH co-localizes with the glutamate transporter VGluT1, confirming its presence in glutamatergic terminals. Following status epilepticus in the transgenic mice, larger sized mossy fiber boutons were found in the dentate inner molecular layer. In acute hippocampal slices of mice that had undergone status epilepticus, a stimulus train delivered to the mossy fiber pathway evoked a robust and rapid SpH fluorescence rise in area CA3 compared with controls, suggesting an increased rate of vesicle release. Furthermore, enhanced endocytotic recycling of vesicles was shown by the faster decay of fluorescence in mice that had experienced status epilepticus.

Finally, in Sprague-Dawley rats, transmission electron microscopy was used to examine ultrastructural changes in presynaptic boutons following status epilepticus. After pilocarpine, mossy fiber boutons were significantly larger and contained many more active zones (release sites) per bouton, suggesting that the area devoted to vesicle release had expanded after status epilepticus. These findings confirm studies documenting increased mossy fiber bouton area following pilocarpine-induced status epilepticus (3). Of interest, the increased bouton area persisted for at least 1 month following pilocarpine status, whereas in the kindling model of epileptogenesis, these plastic changes were transient, lasting only a few days (3). Therefore, persistent structural plasticity of mossy fiber presynaptic terminals appears to be model dependent.

Despite the varied methods and the use of two different rodent models, the results obtained in this study are complementary and consistent—status epilepticus alters presynaptic function and structure in the direction that favors increased release of excitatory transmitter and hence predisposes to hyperexcitable circuit function. Future studies might correlate the extent of these changes with the number and severity of spontaneous recurrent seizures, measures that were not assessed in these experiments. It would also be informative to examine short-term changes in presynaptic plastic changes immediately following status epilepticus, as well as the effects of shorter and less severe seizures.

These results add another dimension to the anatomic and physiologic basis of hippocampal hyperexcitability underlying limbic epilepsy. In addition to the increased excitatory circuit activity engendered by mossy fiber sprouting and changes in intrinsic excitability of dentate granule cells, hilar neurons, and CA3 pyramidal neurons, this study provides strong evidence for altered presynaptic release mechanisms as well. It seems that the post–status epilepticus hippocampus is impaired both coming and going—from both the presynaptic side and the postsynaptic side. The epileptic animal might well have changed its tune to "please release me…and let me *seize* again"! Yet, with every challenge comes an opportunity, and with this additional target, it might be possible to develop therapeutic interventions that compensate for the presynaptic pathophysiology.

by Carl E. Stafstrom, MD, PhD

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