

## QUESTIONING THE ROLE OF ZINC IN EPILEPTOGENESIS

### Lack of Effect of Mossy Fiber-Released Zinc on Granule Cell GABA<sub>A</sub> Receptors in the Pilocarpine Model of Epilepsy

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The recurrent mossy fiber pathway of the dentate gyrus expands dramatically in the epileptic brain and serves as a mechanism for synchronization of granule cell epileptiform activity. It has been suggested that this pathway also promotes epileptiform activity by inhibiting GABA<sub>A</sub> receptor function through release of zinc. Hippocampal slices from pilocarpine-treated rats were used to evaluate this hypothesis. The rats had developed status epilepticus after pilocarpine administration, followed by robust recurrent mossy fiber growth. The ability of exogenously applied zinc to depress GABA<sub>A</sub> receptor function in dentate granule cells depended on removal of polyvalent anions from the superfusion medium. Under these conditions, 200 μM zinc reduced the amplitude of the current evoked by applying muscimol to the proximal portion of the granule cell dendrite (23%). It also reduced the mean amplitude (31%) and frequency (36%) of miniature inhibitory postsynaptic currents. Nevertheless, repetitive mossy fiber stimulation (10 Hz for 1 s, 100 Hz for 1 s, or 10 Hz for 5 min) at maximal intensity did not affect GABA<sub>A</sub> receptor-mediated currents evoked by photorelease of GABA onto the proximal portion of the dendrite, where recurrent mossy fiber synapses were located. These results could not be explained by stimulation-induced depletion of zinc from the recurrent mossy fiber boutons. Negative results were obtained even during exposure to conditions that promoted transmitter release and synchronized granule cell activity (6 mM [K<sup>+</sup>]<sub>o</sub>, nominally Mg<sup>2+</sup>-free medium, 33°C). These results suggest that zinc released from the recurrent mossy fiber pathway did not reach a concentration at postsynaptic GABA<sub>A</sub> receptors sufficient to inhibit agonist-evoked activation.

### COMMENTARY

Zn<sup>2+</sup> is a divalent cation that is prominently concentrated in synaptic terminals where it is intermixed with vesicles and may also be observed in synaptic clefts. In this study, the authors have investigated the possibility that release of the divalent cation Zn<sup>2+</sup> from synaptic terminals may alter GABA<sub>A</sub> receptor dependent inhibition in the hippocampus. Pharmacological studies have demonstrated that certain subsets of GABA<sub>A</sub> receptors are sensitive to blockade by Zn<sup>2+</sup>. This is potentially an interesting question for epilepsy research, as there is also evidence in epilepsy models that Zn<sup>2+</sup> may modify inhibition acutely in experimental status epilepticus, and in cultures of dissociated granule cells from the dentate gyrus of epileptic rats. Zn<sup>2+</sup> sensitivity of GABA<sub>A</sub> receptors is of particular interest in the hippocampus, where sprouted mossy fiber synaptic terminals containing relatively large amounts of releasable Zn<sup>2+</sup> are observed in the supragranular layer of the dentate gyrus in both experimental models and resected human epileptic hippocampus. Release of Zn<sup>2+</sup> from these sprouted mossy fiber terminals could reduce inhibition in hippocampal circuitry reorganized by preceding seizures, and therefore could play a role in epileptogenesis.

In this physiological study, responses evoked in granule cells of the dentate gyrus from pilocarpine treated rats by application of the GABA<sub>A</sub> agonist muscimol were reduced by 200 μM Zn<sup>2+</sup>, which also reduced the amplitude and frequency of miniature IPSCs. The effects were noted only when polyvalent anions (PO<sub>4</sub><sup>-3</sup>, SO<sub>4</sub><sup>-2</sup>) were removed from the bathing media, presumably because these anions bind Zn<sup>2+</sup> and render it inactive. In the same conditions, repetitive stimulation of mossy fibers in CA3 had no effect on GABA<sub>A</sub> currents evoked by photorelease of caged GABA in the proximal dendritic region of granule cells, which is the site of sprouted mossy fiber terminals. The lack of evidence for Zn<sup>2+</sup> blockade of GABA<sub>A</sub> currents in physiologically relevant conditions suggests that the effects of Zn<sup>2+</sup> may be less dramatic than previously suspected, or may be encountered only in unusual metabolic conditions, e.g., status epilepticus. The study provides another example of how a relatively clearly demonstrated *in vitro* phenomenon, such as the *in vitro* ability of Zn<sup>2+</sup> to block inhibitory currents, may have very conditional or variable effects in a complex *in vivo* system of neural circuitry and epileptogenesis.

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