

GABAERGIC INTERNEURON REORGANIZATION DURING THE LATE PERIOD MAY CONTRIBUTE TO HIPPOCAMPAL EPILEPTOGENESIS

Alterations of Hippocampal GABAergic System Contribute to Development of Spontaneous Recurrent Seizures in the Rat Lithium-Pilocarpine Model of Temporal Lobe Epilepsy

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Reorganization of excitatory and inhibitory circuits in the hippocampal formation following seizure-induced neuronal loss has been proposed to underlie the development of chronic seizures in temporal lobe epilepsy (TLE). Here, we investigated whether specific morphological alterations of the GABAergic system can be related to the onset of spontaneous recurrent seizures (SRS) in the rat lithium-pilocarpine model of TLE. Immunohistochemical staining for markers of interneurons and their projections, including parvalbumin (PV), calretinin (CR), calbindin (CB), glutamic acid decarboxylase (GAD), and type 1 GABA transporter (GAT1), was performed in brain sections of rats treated with lithium-pilocarpine and sacrificed after 24 h, during the silent phase (6 and 12 days), or after the onset of SRS (10–18 days after treatment). Semiquantitative analysis revealed a selective loss of interneurons in the stratum oriens of CA1, associated with a reduction of GAT1 staining in the stratum radiatum and stratum oriens. In contrast, interneurons in CA3 were largely preserved, although GAT1 staining was also reduced. These changes occurred within 6 days after treatment and were therefore insufficient to cause SRS. In the dentate gyrus, extensive cell loss occurred in the hilus. The pericellular innervation of granule cells by PV-positive axons was markedly reduced, although the loss of PV-interneurons was only partial. Most strikingly, the density of GABAergic axons, positive for both GAD and GAT1, was dramatically increased in the inner molecular layer. This change emerged during the silent period, but was most marked in animals with SRS. Finally, supernumerary CB-posi-

tive neurons were detected in the hilus, selectively in rats with SRS. These findings suggest that alterations of GABAergic circuits occur early after lithium-pilocarpine-induced status epilepticus and contribute to epileptogenesis. In particular, the reorganization of GABAergic axons in the dentate gyrus might contribute to synchronize hyperexcitability induced by the interneuron loss during the silent period, leading to the onset of chronic seizures.

COMMENTARY

This study examines the lithium–pilocarpine model of temporal lobe epilepsy. In this model, systemic administration of lithium and pilocarpine induces status epilepticus, which is then followed by a so-called silent period, during which electrographic abnormalities can be seen. The end of that period is marked by the appearance of recurrent spontaneous seizures. Modifications in inhibitory circuits (interneuronal loss) and the expression of GABA transmission (heightened expression GABA synthetic enzyme, glutamic acid decarboxylase [GAD]) occur during this process, but the changes present at the onset of spontaneous recurrent seizures have not been well documented. Therefore, the focus of this work was to examine the modifications in GABAergic circuits occurring during this model's silent period.

To examine the distribution of interneurons and their processes, immunohistochemical markers for the various calcium-binding proteins expressed by these cells (parvalbumin, calretinin, and calbindin), as well as for GAD and the type 1 GABA transporter, were used. Data were obtained from four groups: control, those sacrificed 24 hours after status epilepticus, those sacrificed at 6 and 12 days after status (silent period), and those processed after spontaneous recurrent seizures began. Profound cell loss was seen among CA1 pyramidal neurons and stratum oriens, and the upper blade of the dentate gyrus showed neuronal damage. The hilus showed progressive cell loss reaching 87% by 12 days. In contrast, there was no significant loss of CA3 pyramidal cells, and the lower blade of

the granule cell layer also appeared spared from neuronal loss. GABA transporter staining was moderate and of variable significance in the CA1–CA3 pyramidal cell layer and the hilus and was unchanged or enhanced in the dentate but was profoundly reduced in stratum oriens. Parvalbumin immunoreactivity was markedly lower in stratum oriens, the hilus, and dentate gyrus but was unaffected in CA1–CA3 stratum radiatum and pyramidale. Calretinin staining was reduced throughout CA1–CA3 and the hilus. The number of calbindin-positive interneurons did not change in stratum radiatum and stratum pyramidale, although a decrease was seen in stratum oriens. Strikingly, the number of calbindin-immunoreactive interneurons in the hilus did not decrease. Instead, it was significantly increased in the spontaneous seizure group compared with all groups. These cells were mostly present next to the crest of the granule cell layer and at the border between the granule cell layer and the proximal CA3 area. Their morphology was different compared with calbindin interneurons in the control and 24-hour groups. They had large, strongly stained somata and more numerous and longer dendrites running in the polymorphic cell layer and through the granule cell layer

into the molecular layer. The significance of this may be speculated.

Taken together, these results suggest that changes in GABAergic circuits may contribute to epileptogenesis and to the expression of spontaneous recurrent seizures. Although mossy fiber sprouting has been proposed as contributing to seizure propagation, the seizures that ensue in the lithium–pilocarpine model do not rely on this process. Thus, the major factor may be alterations in the GABA system. Although the loss of GABAergic elements in hippocampus would certainly predispose to hyperexcitability, the appearance of the supernumerary calbindin-positive neurons in the hilus, especially at the onset of clinical seizures, is more intriguing. Increased inhibitory network projections in the hilus may lead to recurrent seizures by hypersynchronization of granule cell activity. Alternatively, new inhibitory afferents may inhibit inhibitory neurons themselves, leading to a release of excitation. Further elucidation of this will require a detailed neurophysiological investigation of this novel circuitry.

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