

LOSS OF GABAERGIC INTERNEURONS IN SEIZURE-INDUCED EPILEPTOGENESIS

Reduced Inhibition of Dentate Granule Cells in a Model of Temporal Lobe Epilepsy

Kobayashi M, Buckmaster PS

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Patients and models of temporal lobe epilepsy have fewer inhibitory interneurons in the dentate gyrus than do controls, but it is unclear whether granule cell inhibition is reduced. We report the loss of γ -aminobutyric acid (GABA)ergic inhibition of granule cells in the temporal dentate gyrus of pilocarpine-induced epileptic rats. In situ hybridization for GAD65 messenger RNA (mRNA) and immunocytochemistry for parvalbumin and somatostatin confirmed the loss of inhibitory interneurons. In epileptic rats, granule cells had prolonged excitatory postsynaptic potentials (EPSPs), and they discharged more action potentials than did controls. Although the conductances of evoked inhibitory postsynaptic potentials (IPSPs) recorded in normal artificial cerebrospinal fluid (aCSF) were not significantly reduced, and paired-pulse responses showed enhanced inhibition of granule cells from epileptic rats, more direct measures of granule cell inhibition revealed significant deficiencies. In granule cells from epileptic rats, evoked monosynaptic IPSP conductances were less than 40% those of controls, and the frequency of GABA_A receptor-mediated spontaneous and miniature IPSCs (mIPSCs) was less than 50% that of controls. Within 3 to 7 days after pilocarpine-induced status epilepticus, miniature IPSC frequency had decreased, and it remained low, without functional evidence of compensatory synaptogenesis by GABAergic axons in chronically epileptic rats. Both parvalbumin- and somatostatin-immunoreactive interneuron numbers and the frequency of both fast- and slow-rising GABA_A receptor-mediated mIPSCs were reduced, suggesting that loss of inhibitory synaptic input to granule cells occurred at both proximal/somatic and distal/dendritic sites. Reduced granule cell inhibition in the temporal dentate gyrus preceded the onset of spontaneous re-

current seizures by days to weeks, so it may contribute, but is insufficient, to cause epilepsy.

Spontaneous Seizures and Loss of Axo-axonic and Axo-somatic Inhibition Induced by Repeated Brief Seizures in Kindled Rats

Sayin U, Osting S, Hagen J, Rutecki P, Sutula T

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Repeated brief seizures evoked by kindling progressively increase seizure susceptibility and eventually induce spontaneous seizures. Previous studies demonstrated that the initial seizures evoked by kindling increase paired-pulse inhibition at 15- to 25-millisecond interpulse intervals in the dentate gyrus and also induce apoptosis, progressive neuronal loss, mossy fiber sprouting, and neurogenesis, which could potentially alter the balance of excitation and/or inhibition and modify functional properties of hippocampal circuits. In these experiments, paired-pulse inhibition in the dentate gyrus was reduced or lost after approximately 90 to 100 evoked seizures in association with emergence of spontaneous seizures. Evoked inhibitory postsynaptic currents (IPSCs) examined by single-electrode voltage-clamp methods in granule cells from kindled rats experiencing spontaneous seizures demonstrated altered kinetics (reductions of ~48% in 10% to 90% decay time, ~40% in τ , and ~65% in charge transfer) and confirmed that decreased inhibition contributed to the reduced paired-pulse inhibition. The loss of inhibition was accompanied by loss of subclasses of inhibitory interneurons labeled by cholecystokinin and the neuronal γ -aminobutyric acid (GABA) transporter GAT-1, which project axo-somatic and axo-axonic GABAergic inhibitory terminals to granule cells and axon initial segments. Seizure-induced loss of interneurons providing axo-somatic and axo-axonic inhibition may regulate spike output to pyramidal neurons in CA3 and could play

an important role in generation of spontaneous seizures. The sequence of progressive cellular alterations induced by repeated seizures, particularly loss of GABAergic interneurons providing axo-somatic and axo-axonic inhibition, may be important in the development of intractable epilepsy.

Loss of Interneurons Innervating Pyramidal Cell Dendrites and Axon Initial Segments in the CA1 Region of the Hippocampus Following Pilocarpine-induced Seizures

Dinocourt C, Petanjek Z, Freund TF, Ben-Ari Y, Esclapez M

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In the pilocarpine model of chronic limbic seizures, vulnerability of γ -aminobutyric acid (GABA)ergic interneurons to excitotoxic damage has been reported in the hippocampal CA1 region. However, little is known about the specific types of interneurons that degenerate in this region. To characterize these interneurons, we performed quantitative analyses of the different populations of GABAergic neurons labeled for their peptide or calcium-binding protein content. Our data demonstrate that the decrease in the number of glutamic acid decarboxylase (GAD) messenger RNA (mRNA)-containing neurons in the stratum oriens of CA1 in pilocarpine-treated rats involved two subpopulations of GABAergic interneurons: interneurons labeled for somatostatin only (O-LM and bistratified cells) and interneurons labeled for parvalbumin only (basket and axo-axonic cells). Stratum oriens interneurons labeled for somatostatin/calbindin or somatostatin/parvalbumin were preserved. The decrease in number of somatostatin- and parvalbumin-containing neurons was observed as early as 72 hours after the sustained seizures induced by pilocarpine injection. Many degenerating cell bodies in the stratum oriens and degenerating axon terminals in the stratum lacunosum-moleculare were observed at 1 and 2 weeks after injection. In addition, the synaptic coverage of the axon initial segment of CA1 pyramidal cells was significantly decreased in pilocarpine-treated animals. These results indicate that the loss of somatostatin-containing neurons corresponds preferentially to the degeneration of interneurons with an axon projecting to stratum lacunosum-moleculare (O-LM cells) and suggest that the death of these neurons is mainly responsi-

ble for the deficit of dendritic inhibition reported in this region. We demonstrate that the loss of parvalbumin-containing neurons corresponds to the death of axo-axonic cells, suggesting that perisomatic inhibition and mechanisms controlling action-potential generation also are impaired in this model.

COMMENTARY

Two key hypotheses pertaining to temporal lobe epilepsy have been that epileptogenesis is due to a reduction of γ -aminobutyric acid (GABA)-mediated inhibition and that this reduction in inhibition arises from the death of GABAergic interneurons. In support of these theories, several immunohistochemical studies have provided evidence that the epileptogenic zone has a reduced number of GABAergic interneurons. However, it has been argued that neuron damage in mesial temporal sclerosis results primarily from the loss of glutamatergic *principal* neurons rather than GABAergic *interneurons*. Furthermore, whereas some research performed on epileptic animals and humans has produced evidence of decreased inhibition in diverse cortical areas, other reports have found relatively normal or even enhanced GABAergic inhibition, during various stages of the epileptogenic process. Other research indicates that altered GABAergic inhibition could arise from mechanisms other than neuronal loss. Many of the electrophysiologic studies, however, have used extracellular stimulation and recording techniques with paired-pulse protocols or similar paradigms, and these approaches may detect large changes in GABAergic inhibition, but they are generally unable to identify the underlying mechanisms. The studies considered in this commentary collectively have focused on the hypotheses that GABAergic neurons are killed after repetitive seizures, and specific subsets of the interneuron population are preferentially destroyed.

Two studies, one by Dinocourt et al. and the other by Kobayashi and Buckmaster, used the pilocarpine-treated rat as a model of temporal lobe epilepsy. Dinocourt et al. analyzed interneuron loss in the CA1 region of the hippocampus, and Kobayashi and Buckmaster looked at interneuron loss in the dentate gyrus. The two studies provide independent lines of evidence that specific groups of GABAergic interneurons, including somatostatin and parvalbumin neurons, are lost shortly after severe status epilepticus. Because the number of GABAergic interneurons also was reduced many months later, the loss appears to be permanent. The combination of immunohistochemical, ultra structural, and electrophysiologic data provide converging evidence that selective loss of specific classes of interneurons results in a functional alteration in inhibition.

The approach used in the third article, by Sayin et al., involved the kindling model of temporal lobe epilepsy. This

study also provided several lines of anatomic and electrophysiologic evidence of a specific and selective loss of particular classes of GABAergic interneurons, but this loss occurred after approximately 100 kindled seizures. In the two studies that used the pilocarpine model, interneuron death was induced by seizures that occurred during convulsive status epilepticus. During the kindling process, however, individual seizures that were electrically evoked led to the death of specific types of interneurons, and the interneuron loss was associated with the development of recurrent spontaneous seizures. The results support the hypothesis that the loss of GABAergic interneurons plays a role in the transition from the kindled state of increased seizure susceptibility, but without spontaneous seizures, to a subsequent condition in which spontaneous seizures actually occur.

Thus all three studies, with multiple independent techniques, demonstrated that epileptic tissue is associated with a reduction in the number of specific GABAergic interneurons—at least when the epileptic tissue generates spontaneous chronic seizures after previously evoked seizures. Strengths of these studies include *quantitative analyses* of miniature inhibitory postsynaptic currents and GABAergic neurons. Although other studies have used these approaches, the collective data in these articles point directly to a partial and selective loss of interneurons. It is not clear why previous studies, by using extracellular stimulation and paired-pulse techniques, have sometimes revealed either no apparent loss of inhibition or even enhanced inhibition. One hypothesis is that interneurons connect to other interneurons, with the loss of one class of interneurons reducing inhibitory input to the remaining interneurons, which leads to an increase in the rate of spontaneous action potentials in these remaining interneurons. If true, experiments based on extracellular stimulation may be misleading because of an overall change in the tone of the inhibitory system. Previously, investigators emphasized that subtle changes in the GABAergic system can have important but veiled effects on the network proper-

ties of cortical systems—a concept that would seem consistent with the data and conclusions in these studies concerning an epilepsy-associated loss of specific types of interneurons.

An important issue raised by the studies (particularly by the work of Kobayashi and Buckmaster) is the need to consider differences in dorsal and ventral parts of the hippocampus. Many *in vivo* experiments in rodents are performed in the dorsal half of the hippocampus because it is accessible and, therefore, more easily studied. However, the ventral hippocampus of the rodent is the region most similar to the anterior hippocampus of humans. In humans, the anterior hippocampus, typically, is the focus of and, thus, the main area of resection for temporal lobe epilepsy. Therefore, experimental studies using either electrophysiologic or anatomic approaches might give greater consideration to this anatomic issue. In addition, experimental models that involve systemic injection of convulsant drugs, such as pilocarpine and kainate, presumably result in widespread damage of the brain. Therefore, it is important to assess how different brain areas contribute to the generation of various types of seizures in each model. Finally, even experiments with repetitive electrical stimulation probably cause damage in many brain areas and, taking into account the impact of this damage, may provide clues to the mechanisms potentially important to epileptogenesis.

The loss of inhibitory interneurons could lead to the unmasking of other mechanisms that may be induced or up-regulated during chronic epileptogenesis, such as *N*-methyl-D-aspartate (NMDA) receptors, neuronal gap junctions, and recurrent excitatory circuits. Thus another important result of these studies may be that they highlight the need to consider the integration of several different mechanisms underlying the functional and structural pathology of injury-induced epileptogenesis.

by F. Edward Dudek, Ph.D.,
and Li-Rong Shao, M.D.