MECHANISM OF EPILEPSY¹

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ABSTRACT

Epilepsy is a collection of diverse disorders that together affect approximately 1% of the general population. Current therapies are largely symptomatic and are aimed at controlling seizures in affected individuals. This review focuses on emerging insights into mechanisms underlying the most common form of epilepsy—complex partial epilepsy—and also addresses progress in molecular genetic approaches. Such developments will hopefully lead to more effective therapies.

INTRODUCTION

Epilepsy is a common neurological disorder, affecting more than two million people of all age groups in the US alone (1). Current medical therapy is largely

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symptomatic and not always satisfactory. Antiepileptic drugs control seizures in many patients, but patients can be kept seizure-free in less than half of the partial epilepsies (2). Removal of epileptic tissue can cure a select population, and new drugs such as felbamate, lamotrigine, and gabapentin are forthcoming, but for many of the epilepsies, we have no effective prophylactic regimen or easy cure. Advances in the knowledge of the mechanisms of epilepsies would allow for more rational therapeutic approaches to this difficult neurological disorder.

This review provides an overview of the current knowledge of the basic mechanisms of epilepsy, based on recent progress in the field. We first discuss briefly the terminology and classification of epileptic seizures, then the recent developments in the understanding of the mechanisms of temporal lobe epilepsy. Lastly, we survey the emerging power of genetics and molecular biology for the study of epilepsy in humans. Space constraints preclude discussions of other promising areas of epilepsy research, including cellular mechanisms of partial seizures and absence seizures.

TERMINOLOGY AND EPILEPTIC SEIZURE CLASSIFICATION

The term seizure refers to a transient alteration of behavior due to abnormal, synchronized, and repetitive burst firing of neuronal populations in the central nervous system (CNS). Epilepsy is a syndrome of episodic brain dysfunction characterized by recurrent unpredictable spontaneous seizures. Partial seizures begin in a localized brain region, whereas generalized seizures show widespread involvement of both hemispheres from the outset (3). Examples of generalized seizures are absence (petit mal), myoclonic, or tonic-clonic (grand mal) seizures. Although a simple partial seizure does not affect the level of consciousness, a complex partial seizure is associated with impairment of consciousness. Most complex partial seizures originate from the temporal lobe and hence are called temporal lobe seizures. Patients frequently have more than one kind of seizure. When simple partial seizure precedes a complex partial seizure, it is referred to as an aura. More recent classification of epileptic syndromes incorporates such features as etiology and age of onset in addition to the different combinations of seizures (4). Other commonly used terms include ictal (of seizure itself) and interictal (between seizures). Convulsion implies ictal behavior with vigorous motor activities. Status epilepticus denotes a very prolonged seizure or seizures occurring so frequently that full recovery of brain function does not occur interictally.
MECHANISMS OF TEMPORAL LOBE EPILEPSY

Complex partial seizures constitute a major percentage of epilepsies and are rather disabling as a result of impaired consciousness. They are often medically intractable in that doses of medications with tolerable side effects will not satisfactorily control the seizures. Most cases of complex partial epilepsy appear to stem from an abnormality intrinsic to the temporal lobe, since partial resection of the temporal lobe, including the mesial structures, hippocampus, and amygdala, virtually eliminates seizures in more than 80% of selected patients (5–8). Histologic examination of the surgical specimens and autopsy studies of patients with chronic temporal lobe epilepsy most often reveal sclerosis of the hippocampus, termed Ammon’s horn sclerosis, which is characterized by a marked loss of the principal neurons of hippocampus and is accompanied by gliosis (9, 10).

Ammon’s Horn Sclerosis: Cause and Effect of Temporal Lobe Epilepsy?

Experimental work has demonstrated that repeated or prolonged seizures (status epilepticus) are sufficient to cause hippocampal sclerosis, presumably through excessive activation of excitatory glutamate receptors, which results in excitotoxicity (11–13). Resection of sclerotic hippocampus leads to dramatic improvement or even a cure of the epileptic condition in humans, which suggests that the sclerotic hippocampus somehow causes the epilepsy. Patients with sclerotic hippocampus often have a history of complicated febrile seizures, i.e. repeated or intense febrile convulsions, or status epilepticus in childhood, followed by later development of temporal lobe epilepsy (10, 14). One could argue, however, that these individuals become susceptible to complicated febrile convulsions or status epilepticus following a perinatal hypoxic injury (perhaps subclinical) that may have already caused hippocampal sclerosis. Yet in the absence of definite known perinatal insult, it seems equally plausible that intense seizures can cause hippocampal sclerosis, which, once developed, can cause epilepsy.

How might the neuronal death and gliosis with accompanying functional and morphologic rearrangements lead to focal hyperexcitability in the hippocampus and the subsequent emergence of epilepsy? Repeated intense seizures cause a loss of recurrent, γ-amino butyric acid (GABA)–mediated inhibition of dentate granule cells in vivo (11). Thus until recently, the leading hypothesis was that death of GABAergic inhibitory interneurons resulted in attenuation of inhibition, which in turn led to pathologic hyperexcitability of the remaining principal neuronal populations—pyramidal and dentate granule neurons—of the hippocampus. However, detailed immunohistochemical studies of the sclerotic hippocampus isolated from experimental models and from humans have
shed new light on the potential mechanisms of hyperexcitability. In work that contradicted the prevailing idea at the time, Sloviter (15) demonstrated in an experimental model that GABA immunoreactive neurons were more resistant to seizure-induced neuronal death than were other hippocampal neurons. Furthermore, immunohistochemistry confirmed the relative preservation of presumed GABAergic interneurons in surgical specimens from humans with epilepsy (16). In contrast to the resistant GABAergic neurons, mossy cells and somatostatin/neuropeptide Y-immunoreactive neurons in the dentate hilus were extremely sensitive to seizure-induced neuronal death in the experimental model.

Mossy cells (not to be confused with mossy fibers, which are the axons of dentate granule cells) constitute the most numerous neuron type in the hilus of the dentate gyrus of hippocampus (17). They receive synaptic inputs both from dentate granule cells (via mossy fibers) and from the entorhinal cortex (via the perforant path, the major afferent pathway for the hippocampus) (18). Mossy cells project both ipsilaterally and contralaterally to the molecular layer of the dentate gyrus where the dendrites of granule cells are located (19, 20). Functionally, mossy cells are activated by perforant path stimulation at a lower threshold than dentate granule cells and are presumably excitatory on their targets (21). Mossy cells are damaged following intense synaptic activation, probably through excitotoxic mechanisms by activation of N-methyl-D-aspartate (NMDA) subtype of glutamate receptors, resulting in excessive rise of intracellular calcium (22).

The preservation of GABAergic neurons in the face of loss of GABA-mediated inhibition of dentate granule cells after intense seizures is paradoxical. This paradox led to the “dormant basket cell” hypothesis (23), which suggests

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*Figure 1*  Schematics of alternative hypotheses explaining hyperexcitability of dentate granule cells. Bottom contains typical field potential recordings from dentate hilus following paired single shocks (denoted by arrows) of perforant path at intervals of 40 msec. In naive animals, the first shock evokes an upgoing excitatory postsynaptic potential (EPSP) interrupted by sharp downgoing event, reflecting synchronous action potentials of populations of granule cells, but the second shock fails to trigger the action potentials, partially owing to GABAergic recurrent inhibition activated by the first pulse. In epileptic animals, both the first and second shocks evoke multiple population action potentials.

Top figures illustrate the “dormant basket cell” hypothesis. Top left shows a simplified normal circuitry in which mossy cells (M) provide excitatory input to both GABAergic basket cells (B) and granule cells (G). Top right depicts how removal of the excitatory input to the basket cells due to seizure-induced death of mossy cells would result in dormancy of basket cells and increased response of granule cells to excitatory synaptic input.

Middle figures explain mossy fiber sprouting. Middle left is the same as top left. Middle right depicts the formation of recurrent excitatory synapse of granule cell axon (mossy fiber) onto its own dendrite following removal of mossy cell input due to seizure-induced death. Formation of these recurrent excitatory synapses is postulated to account for the increased response of granule cells to excitatory synaptic input. Reprinted with permission from the *Journal of Neuroscience*. 
that the seizure-induced death of excitatory neurons in the hilus (probably mossy cells) removes a tonic excitatory projection to GABAergic basket cells, the inhibitory neurons in the dentate gyrus, resulting in a disinhibition because basket cells lie dormant when they are not activated by mossy cells (Figure 1). Once initiated, one can easily imagine a vicious cycle in which a partial loss of this inhibition, combined with excitatory synaptic input due to otherwise phys-
iologic stimuli, could lead to excessive firing of granule cells, more mossy cell death, further loss of GABAergic inhibition, and so on, ultimately resulting in the emergence of an epileptic condition long after the initial injury. In an experimental model of unilateral hippocampal sclerosis from focal electrical status epilepticus, the resulting hyperexcitability of hippocampal neurons was normalized by stimulation of the unaffected contralateral hippocampus (23). One obvious explanation in support of this hypothesis is that dormant basket cells were awakened by preserved contralateral mossy cells projecting across through the hippocampal commissure.

An alternative to the “dormant basket cell” hypothesis is the possibility that hyperexcitability of dentate granule cells is a consequence of a pathologic neuronal rearrangement in which excitatory granule cells innervate themselves, resulting in a recurrent excitatory circuit and another vicious cycle. This hypothesis proposes that death in the hilus of neurons that normally project to the dendrites of the granule cells results in loss of synaptic contacts, which are replaced by mossy fibers of granule cells themselves, presumably by the process of sprouting (Figure 1). These fibers contain high concentrations of zinc, which can be readily identified by Timm staining, thereby facilitating detection of axonal rearrangements of dentate granule cells. A dramatic increase in this projection in the molecular layer of the dentate gyrus has been demonstrated following seizures induced by kainate, a neurotoxin (24). Similar findings were obtained in the kindling model of epilepsy (25) and in surgical specimens from medically intractable epileptic patients (26). The usual paired pulse inhibition (physiological indicator of recurrent GABAergic inhibition) from normal animals was not only eliminated but was changed to paired pulse facilitation in animals treated with kainate with robust sprouting (24). This observation supports the argument that the observed hyperexcitability is not simply a reduction of inhibition but an increase in excitation and thus evidence for the emergence of functional recurrent excitatory synapses.

Despite histologic demonstration of mossy fiber sprouting, the targets of these sprouting projections have yet to be determined. Furthermore, functional consequences of these aberrant projections remain controversial. In dentate granule cells of kainate-treated rats, reduction of inhibition and increased excitability precedes the development of histologically demonstrable mossy fiber sprouting (27). The time course of development of mossy fiber sprouting correlates more with recovery of recurrent inhibition than with increased excitability. These findings suggest that the net effect of the sprouting is actually inhibitory, perhaps as a result of preferential reinnervation of GABAergic basket cell dendrites left bare by mossy cell death. Mossy fiber sprouting may therefore represent a compensatory defensive response to the developing hyperexcitability.
It is possible that both hypotheses may be correct and that they cooperate to promote epileptogenesis. In hippocampal slices in vitro isolated from kainate-treated rats, dentate granule cells in normal media exhibit relatively normal electrophysiologic responses (28). However, in the presence of bicuculline, which blocks GABAergic inhibition, antidromic hilar stimulation results in synchronous bursts of granule cells in some of the slices from kainate-treated animals with histologically robust mossy fiber sprouting but in none from those without robust sprouting and in none from control animals. It has therefore been suggested that both inhibitory and excitatory circuits are newly formed, but the normally suppressed excitatory connections emerge when synaptic inhibition is blocked.

Although many questions remain unanswered, plausible hypotheses continue to be formed and tested anatomically and physiologically. The results will allow further insight into how these selective neuronal injuries and deaths may lead to the vicious cycle of reduction of inhibition and enhancement of excitation, which in turn causes further damage, ultimately resulting in spontaneous occurrence of seizures.

The other half of this vicious cycle is also of tremendous interest, i.e. what is the molecular mechanism underlying these seizure-induced cell deaths? Although the excitotoxicity is the most likely mechanism with participation of the NMDA subtype of glutamate receptors and intracellular calcium, subsequent calcium-activated intracellular signaling pathways leading to neuronal death remain obscure. However, seizures induce the expression of immediate early genes such as c-fos in most hippocampal neurons and, with repeated severe seizures, c-fos is induced biphatically with a delayed time course in some hippocampal neurons destined to die (29). This latter observation suggests that gene expression may contribute to the death of neurons because many immediate early genes code for transcription factors, which in turn regulate expression of target genes. If this is the case, identification of the death genes and of the intracellular signals controlling their expression becomes critical. Elucidating the intracellular signaling pathways linking glutamate receptor activation to transcriptional activation of c-fos may provide further understanding of these underlying molecular mechanisms (30, 31).

If mossy fiber sprouting does contribute to epileptogenesis, what is the molecular basis of sprouting? Many neurotrophic factors are involved in the general process of sprouting. Genes encoding neurotrophins [nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF)] (32, 33) and neurotrophin receptors (TrkB and TrkC) (34) in the dentate granule cells and in multiple additional neuronal populations are induced by seizures. Perhaps the molecular machinery required for morphologic arrangements and rearrangements that function principally in developing brain could be recruited by seizures for adaptive or maladaptive processes such as mossy fiber sprouting.
Answers to these questions will have significant therapeutic implications. Insight into the molecular basis of seizure-induced neuronal death could lead to preventive therapies aimed at protecting neurons following intense seizures or complicated febrile convulsions in children. Identification of the key molecular components of mossy fiber sprouting may allow for specific pharmacologic intervention with the goal of enhancing adaptive responses or limiting maladaptive responses. Selective pharmacologic means of activating dormant basket cells may lead to a new class of antiepileptic agents.

**GENETIC APPROACHES TO EPILEPSY**

We have seen remarkable progress in the understanding of the mechanisms underlying temporal lobe epilepsy. In contrast, the power of genetics and molecular biology is just beginning to be used to clarify the molecular basis of inherited epilepsies. Although inherited forms are estimated to account for only 20% of all epilepsies, genetic predisposition may influence the development of acquired epilepsy in individuals with brain injury. Thus knowledge of the molecular basis of inherited epilepsies could provide important insight into the mechanisms of epilepsy in general.

Shoffner et al were the first to identify the genetic defect in a human epileptic syndrome (35). Consistent with maternal inheritance, a mutation of a mitochondrial gene encoding a mitochondrial tRNA coding for lysine was found to be responsible for a condition characterized by myoclonic epilepsy and by a myopathy with a distinctive histochemical abnormality referred to as ragged-red fibers. This disease is known as the myoclonic epilepsy and ragged-red fibers (MERRF) syndrome and is associated with defects in mitochondrial oxidative phosphorylation. The mitochondrial abnormality in the appropriate brain areas with its deleterious effect on the aerobic metabolism will presumably lead to neuronal dysfunction underlying the myoclonic seizures.

Linkage analyses have successfully identified the chromosomal localizations of mutant genes underlying three different familial epilepsies: benign neonatal convulsions (20q); progressive myoclonic epilepsy (21q22.3); and tuberous sclerosis (9q34 and 11q23 in different kindreds). In juvenile myoclonic epilepsy, localization to 6p remains controversial pending further verification (36). The emerging success of positional cloning in detecting the responsible gene in diseases such as cystic fibrosis, neurofibromatosis, myotonic dystrophy, and Huntington’s disease offers hope for similar successes with the epilepsies.

In an alternative approach, identification of candidate genes may expedite detection of the mutant genes. This approach exploits the rapid progress in the molecular cloning of cDNAs encoding receptors and ion channels that regulate neuronal excitability. Cloning of a particular candidate cDNA enables the
physical localization of the homologous human gene to a particular region of a human chromosome. If the physical localization of the candidate gene corresponds to the genetically determined locus of an inherited epilepsy identified by linkage analysis, the locus and the candidate gene can be more extensively evaluated.

Two classes of genes that may be candidates are glutamate receptor and potassium channel genes, both of which are intimately involved in the regulation of neuronal excitability. Molecular cloning of members of each class is proceeding rapidly. The human chromosomal localization of these genes is just beginning to emerge with the localization of genes (GluR1–5) encoding the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subtype of glutamate receptor to human chromosomes 5q32-33, 4q32-33, Xq25-26, 11q22-23, and 21q21.1-22.1, respectively (37, 38). The GluR4 maps to a region that is associated with tuberous sclerosis, a disease that often involves seizures, in some families, as mentioned above. Identification of polymorphism in these candidate genes in various pedigrees will enable investigators to assess whether they may be relevant in epilepsy.

Continuing developments in basic neuroscience will further guide and complement human genetic approaches. For example, analysis of structure-function relationship has been performed with glutamate receptor channel ionic selectivity (39) and potassium channel inactivation (40) using site-directed mutagenesis of cDNAs and expression system. This analysis has enabled the identification of specific base pairs at which mutations could drastically modify channel function, thereby resulting in neuronal hyperexcitability or altered synaptic responses. Identifying and characterizing the regulation of the relevant neurotransmitter receptor and ion channel function by second and tertiary messengers will provide additional candidate genes. The rapid pace of chromosomal mapping of DNA in the Human Genome Project offers considerable promise of progress in this line of inquiry.

CONCLUDING REMARKS

The last several years have witnessed remarkable advances in our knowledge of the cellular and molecular mechanisms of the epilepsies. In contrast to a homogeneous neurological disorder such as Huntington’s Disease, epilepsy comprises an enormous heterogeneity of disorders. Considering the complexity of the disease, progress has been truly extraordinary. Plausible explanations for the mechanisms of development of neuronal hyperexcitability in acquired epilepsies have been put forth, leading to hypotheses that can be tested with available cellular and molecular techniques. The etiology of a human inherited epilepsy has been identified through a molecular genetic approach. Insights derived from diverse approaches to epilepsy ranging from cellular electrophys-
iology to molecular genetics will be complementary and mutually reinforcing. In coming years, these emerging discoveries should lead to more rational strategies and novel therapeutic approaches based upon a better understanding of the disease mechanisms.

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