The process of epileptogenesis: a pathophysiological approach

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Several recent advances have contributed to our understanding of the processes associated with mesial temporal lobe epilepsy in humans and in experimental animal models. Common pathological features between the human condition and the animal models may indicate a fundamental involvement of the given pathology in the process of epileptogenesis.

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Abbreviations

GAD	glutamate amino decarboxylase
GABA	γ-aminobutyric acid
GluR	glutamate receptor
IPSC	inhibitory postsynaptic currents
MTLE	mesial temporal lobe epilepsy
NMDA	*N*-methyl-D-aspartate
SE	status epilepticus

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Introduction

New insights into the role of mesial temporal lobe epilepsy (MTLE) in humans and in animal models have unravelled specific changes underlying epileptogenesis and specific events related to seizures. Whereas other or more general fields of basic epilepsy research have been covered by recent reviews [1,2], the present review will deal with recent progress in our understanding of the pathologies involved in MTLE.

As epilepsy is considered to involve hyperexcitable neurons, a basic assumption in epilepsy research links the pathogenesis of epilepsy and the generation of synchronized neuronal activity (seizures) with an imbalance between inhibitory [γ-aminobutyric acid (GABA)-mediated] and excitatory (glutamate-mediated) neurotransmission in favor of the latter. MTLE frequently results from an initial precipitating injury, which predisposes individuals to aggravating seizures and hippocampal sclerosis at later stages. Although the specific pathology of the initial precipitating injury is unclear, the final stages of MTLE can be studied after surgical resection of the epileptic focus. In laboratory rodents, status epilepticus (SE)-inducing insults such as continuous perforant path stimulation or the administration of pilocarpine or kainate have been shown to produce a condition with spontaneous limbic seizures and hippocampal sclerosis that closely mimics clinical findings. Despite being based on quite a different etiology, similarities in the pathology between species and experimental models are likely to result from common mechanisms underlying the generation of spontaneous seizures in man and animal models. Cross-correlation studies of human and animal data over the past decade have extended our knowledge on the similarities and discrepancies between basic science and clinical findings, underscoring the predictive value of certain animal models to further our understanding of human MTLE.

Morphological changes in mesial temporal lobe epilepsy

Hippocampal sclerosis is specific for MTLE in humans [3], and is characterized by tissue shrinkage, cell loss and reactive gliosis in all hippocampal subfields as well as in the entorhinal cortex. Neuronal losses involve the hilar mossy cells, hilar somatostatin-containing interneurons and CA1 pyramidal cells, whereas hilar and CA1–3 glutamate amino decarboxylase (GAD)-positive interneurons are relatively spared [4,5]. The granule cell layer is frequently dispersed, presumably because of the concerted action of brain-derived neutrotrophic factor

and trkB. Axons of the granule cells, interneurons and surviving CA1 pyramidal cells sprout and establish functional aberrant neuronal circuits [4–6]. Animal models employing SE-inducing insults as the initial precipitating injury including continuous perforant path stimulation, kainate or pilocarpine administration to rats, after 4–6 weeks (the silent or latent period) produce a condition of recurrent spontaneous seizures and hippocampal pathology with a high degree of similarity to that found in human MTLE. Common traits between rat models include an approximately 50% loss of hilar neurons, mostly mossy cells and somatostatin (SS)-positive interneurons, and a loss of CA1 horizontal SS/GAD-positive interneurons of the stratum oriens and alveus [7–10]. Losses of CA1–3 pyramidal cells are variable, depending on the species, strain and chemoconvulsant model [8,10,11•]. Granule cells are well preserved (in rats) and mossy fibers sprout into the supragranular layer and into the CA3 stratum oriens, but this is not necessarily correlated with seizure severity [8,12]. GABAergic fibers also sprout in the dentate and in the CA1 [13•], whereas CA1 pyramidal neurons sprout to form recurrent excitatory feed-forward local circuits [14•]. The findings in rat models are at odds with a recently developed model of MTLE in mice, in which kainate injection into the CA1 region produces no silent period, a rapid loss of hilar interneurons, a delayed loss of CA1 pyramidal cells, and granule cell dispersion [11•].

The neuronal loss and synaptic restructuring in models with SE as the initial precipitating injury is much more extensive than that observed after kindling [15], possibly because of frequent spontaneous seizures in the former. However, the variability in the epileptic phenotype questions the relevance of model-specific traits for epileptogenesis. For example, an extensive loss of CA3 pyramidal cells causing a significant deafferentiation of the CA1 is invariably seen after kainate, but not pilocarpine administration in rats. Likewise, the moderate loss of CA1–3 pyramidal cells in the pilocarpine model can almost be completely prevented by kindling before pilocarpine-induced SE, with no impairment in the development of spontaneous seizures [16••]. Together with the use of fast- or slow-kindling rats [17], these examples illustrate the importance of using animals with different pheno- or genotypical backgrounds. Changes less relevant to epileptogenesis are thus more likely to be filtered out by comparing findings between animal models. This approach is useful for determining whether certain findings are pivotal for epileptogenesis, or whether they are merely by-products of epileptogenesis, or conversely may constitute protective mechanisms against seizures. The goal of such comparisons should be the identification and characterization of common pathological traits, necessary and sufficient for epileptogenesis in animal models and ultimately in patients with MTLE.

The role of hilar neuronal loss and mossy fiber sprouting

Sprouting is classically seen as a response to the loss of neuronal targets. Accordingly, the loss of mossy cells and SS interneurons in the hilus should lead to sprouting in the inner and outer molecular layers, respectively. It has been clearly established that mossy fibers in humans with MTLE and in animal MTLE models form excitatory recurrent circuits through collaterals synapsing onto granule cell and interneuron dendrites in the supragranular layer and onto new subgranular dendrites in the hilus [8,12,18–20,21•]. The role of a recurrent granule cell excitatory feedback in the gating mechanism of the dentate gyrus will depend on the output function of dentate granule cells. The main targets of the mossy fibers are the two distinct populations of seizure-resistant [8] hilar cholecystokinin- and parvalbumin-containing basket cells [22,23•]. The output from the granule cell–mossy fiber system thus produces a strong activation of GABAergic hilar neurons. Accordingly, activation of the entorhinal cortical input to the dentate leads to granule cell and interneuronal population bursts and the depression of intrahippocampal associational pathways [24]. The few cases with hilar parvalbumin-positive basket cell losses in kainate-treated rats have more pronounced sprouting, loss of granule cell inhibition and more severe epilepsy [8].

Sprouting has been suggested to originate selectively from newly formed granule cells after seizure-induced neurogenesis [25]. However, recent experiments have ruled out this possibility [26•,27]. It is also becoming evident that mossy fiber sprouting is not necessary for epileptogenesis in animal models. Such sprouting occurs after non-epileptogenic seizures in the absence of neuronal loss [28•], and preventing its expression by the protein synthesis inhibitor cycloheximide does not impede epileptogenesis in pilocarpine-treated rats [29]. Mossy fiber sprouting may thus be a phenomenon simply associated with seizures, but not absolutely necessary for epileptogenesis. Other types of abnormal connectivity are also observed during epileptogenesis, including CA1 pyramidal cell axonal sprouting [14•], GABAergic sprouting in the hilus and in the CA3–1 [4,8], transient spine loss on the proximal dendrites of dentate granule cells [30], and possibly the formation of de novo gap junctions in small clusters of neuronal ensembles [31••,32]. The latter findings are of interest because extracellular recordings have revealed high frequency (200–500 Hz) synchronization exclusively in neuronal clusters of the epileptogenic zone [31••].

Interneuron activity in mesial temporal lobe epilepsy and the dormant basket cell hypothesis

The dormant basket cell hypothesis [7] seeks to explain functional consequences of missing excitatory hilar

mossy cells, thought to deprive interneurons of significant excitatory afferents, and leading to a disinhibition of granule cells. Various aspects of the hypothesis have recently been reviewed [33,34], and here we will address the hypothesis in relation to the excitatory input onto interneurons. Approximately 90% of the ipsilateral synaptic contacts formed by mossy cells are made on the spines of proximal granule cell dendrites [35]. The loss of mossy cells may thus deprive basket cells of only a minor excitatory input. Because interneurons already receive a vast excitatory input from existing and presumably sprouted mossy fibers [22], this loss will probably not alter afferent excitatory input onto interneurons. Indeed, paired-pulse experiments and patch clamp recordings show increased or unchanged granule cell inhibition in humans with MTLE and animal models after SE or kindling [8,36,37,38•]. Contrary to the dentate, there is a significant decrease in the frequencies of miniature inhibitory postsynaptic currents (IPSC) recorded in CA1 pyramidal cells of epileptic rats [39•,40], and a borderline significant reduction in paired pulse suppression of associational pathways in rat CA1 and the epileptogenic human hippocampus [36,41]. A group of interneurons in the CA1 oriens and alveus are clearly lost in epileptic rats [10]. However, the density of perisomatic GABAergic synapses onto pyramidal cells [13•,39•] and monosynaptic inhibition of CA1 pyramidal cells is similar in epileptic and control rats [41–43], making it unlikely for CA1 pyramidal cells to become hyperexcitable due to a reduced inhibitory drive. Although there is evidence for reduced excitatory postsynaptic currents (EPSCs) in lacunosum moleculare interneurons in the kainate model upon afferent stimulation [43], it is difficult to perceive why surviving GAD-positive interneurons throughout the hippocampus, including the lacunosum–moleculare, should respond with an increased expression of GAD as a result of dormancy [44]. Increased inhibition in the dentate and hyperexcitability in the CA1 is not consistent with a compromised excitatory drive onto interneurons, but rather with a number of other factors including altered GABA and glutamate receptor (GluR) composition and expression, release properties of transmitters and restructuring of axonal pathways to establish excitatory feed-forward connections.

Altered GABAergic transmission

The original hypothesis about the imbalance between excitation and inhibition in MTLE postulates a decreased GABAergic function. The latest research indicates that this is not necessarily the case. For example, the number of perisomatic GABAergic synapses on CA1 pyramidal cells are unaltered in epileptic rats [13•,39•]. The observed decrease in IPSC frequency may thus be caused by altered presynaptic GABA release. The altered inhibition in MTLE inhibitory characteristics of the dentate and the CA1 also involve changes in the molecular assembly of $GABA_A$ receptors. Increases in $\alpha1$, $\alpha2$, $\alpha3$, $\alpha4$, $\alpha5$, $\beta2$, $\beta3$ and $\gamma2$ $GABA_A$ receptor subunits in dentate granule cells in animal MTLE models (although changes are model-specific, e.g kainate versus pilocarpine) and human epileptic tissues [37,45–47] are thought to underlie the increased frequency and amplitude of miniature IPSC, zinc sensitivity and the shift in benzodiazepine modulation [37,48,49]. Granule cells in hippocampi resected from humans with MTLE also exhibit increased GABA and zinc potency, but unaltered benzodiazepine efficacy [50], which points to a non-similar $GABA_A$ receptor composition in epileptic humans and rats [46]. Distinct populations of granule cells from human epileptic hippocampi express different subunits, shown to underlie differential sensitivity to clonazepam [51]. Hilar interneurons also have higher levels of $\alpha1$, $\alpha5$, $\beta2$, $\beta3$ and $\gamma2$ subunits after kainate treatment, but the functional implications of this are unknown. In contrast to the dentate gyrus, CA1 pyramidal cells exhibit decreased efficacy, increased potency of GABA, an unchanged zinc and a decreased zolpidem sensitivity [49], in line with the compromised inhibition in this area of epileptic human tissue [36] and of rat models [41]. The loss of CA1 pyramidal cells parallels the reduced levels of $\alpha5$, $\beta3$ and possibly $\gamma2$ subunits, whereas CA1 interneuronal expression of $\gamma2$ appears to be unaltered [45].

The zinc-loaded mossy fiber sprouting and the increased zinc sensitivity of GABA receptors has been suggested to undermine GABAergic inhibition [48,52]. However, in the light of the persistence of seizures in the absence of a zinc delivery system onto the altered $GABA_A$ receptors (i.e. lack of mossy fiber sprouting [29]), this hypothesis may need to be revisited.

Even if only region-specific, an apparently enhanced inhibition in MTLE is difficult to reconcile with epileptogenesis. New possibilities for the role of inhibition and interneurons in epilepsy should be considered, as GABAergic interneurons can effectively synchronize neuronal activity [53]. Moreover, granule cells have been shown transiently (<24 h) to express GAD-67 after SE [54]. The significance of this phenomenon to epileptogenesis may be strictly related to SE, but the capacity of granule cells to become GABAergic represents a possibility for the complete restructuring of the normal inhibitory network in response to severe neuronal stimuli. In line with the paradoxical increase of GABA-mediated transmission in MTLE, it is interesting to note that hyperthermia-induced seizures in immature rats (a model of febrile seizures in man) result in a life-long increase in the function of hippocampal GABA synapses [55••], whereas the animals become more prone to epileptogenesis [56•].

Glutamatergic and other excitatory mechanisms in epileptogenesis

Ionotropic GluR are divided into three classes: α-amino-3-hydroxy-5-methyl-isoxazole propionate (AMPA), *N*-methyl-D-aspartate (NMDA) and kainate receptors named after preferred agonists and based on molecular sequence homologies. Metabotropic GluR are classified into three groups (I–III) on the basis of second messenger pathways and pharmacology. The blockade of GABAergic neurotransmission usually uncovers an increased excitatory drive in the hippocampi of epileptic animals and humans [19,20,21•,57]. However, because increased excitatory activity, at least in the dentate, appears to be adequately counterbalanced by inhibition, it is still not clear how the increased gain of excitatory drive dictates neuronal population bursting and epileptic seizures through the hippocampus.

Glutamate release and hence glutamate receptor activation is increased during kindling epileptogenesis and during and after SE. The effect of chronically elevated glutamate levels caused by a lack of glutamate transporter subtypes GLT-1 and GLAST render mice more seizure-susceptible [58,59], but this particular mechanism may be less relevant in the kindling model of epilepsy [60,61]. The correlate of this situation in epileptic tissue is most likely caused by an altered release probability, as has been demonstrated at the mossy fiber–CA3 pyramidal cell synapse, and shown to involve a significantly increased pool of releasable glutamate [62]. The increased NMDA receptor activation in MTLE [63•] is also significant, given the pivotal role of this receptor in plasticity and long-term gene regulation through the persistent (1 year after SE) activation of transcription factor AP-1 [64]. The NMDA receptor antagonist MK-801 has antiepileptogenic effects during kindling, and blocks the expression of spontaneous seizures after SE [65,66], but has no effect against acute kindled or clinically refractory seizures. This suggests that NMDA receptor activation in epilepsy may initiate a cascade of cellular events that later loses its dependency on NMDA receptors. Whereas the effects of MK-801 originally made NMDA receptors unsuitable as therapeutic targets for MTLE therapy, recent progress with low-affinity ligands [67] or the anticonvulsant effects of vaccine-induced inhibition of the NMDA receptor-subunit NR1 [68••] may change this view. Levels of NMDA receptors appear to be increased in epileptic tissue [69–72], but the gain of NMDA receptor-mediated transmission in MTLE is also effectively enhanced by post-translational modifications of NMDA receptor subunits such as phosphorylation [73,74] and redox modulation [75]. Genetically modified mice expressing a Q/R unedited GluR-B subunit to enhance glutamate-induced calcium influx through AMPA receptors suffer from temporal lobe epilepsy [76,77] similar to mice lacking the presynaptic vesicle, SV2A and SV2B [78]. This is in line with an increased calcium influx in epileptic neurons [79–81], leading to the activation of downstream cellular processes responsible for axonal outgrowth and synaptogenesis. Some of the calcium-dependent effects may be counteracted by direct or indirect pharmacological modulation of calcium channels [82–84]. However, the Ca^{2+}-related changes in epileptic tissue are non-equivocal. Some neurons such as the dentate gyrus granule cells may have developed a defence mechanism against excessive Ca^{2+} entry. In human MTLE and in several animal models, the loss of calbindin from these cells results in a decreased Ca^{2+} entry during repetitive firing [85]. Similarly, selective increase of metabotropic GluR4 in granule cells of patients with MTLE [86•] may serve a protective purpose.

Conclusion

The development of new animal models and extensive comparative anatomical and physiological studies with tissue obtained from MTLE patients have pointed out a number of common pathophysiological conditions. Such studies have enabled us to revisit the dormant basket cell hypothesis, and have cast doubt about the necessity of mossy fiber sprouting and certain neuronal losses for epileptogenesis. Future research efforts will have to focus on key factors of epileptogenesis to separate epileptogenetic events from by-products of epileptic seizures or from possible endogenous protective neuronal mechanisms activated to defend against the deleterious effects of epileptic discharges. In this quest it will be extremely important to determine the precise timing of the critical neuronal alterations. We have to keep in mind that the sight of an open airbag in a car accident may seem to the uninitiated to be the cause of the crash rather than a device designed to protect against it.

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