Recent advances in epileptogenesis

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Epileptogenesis, the transformation of the brain to a long-lasting state characterized by recurrent seizures, can occur due to genetic or acquired mechanisms. The mutant genes for three idiopathic syndromes (begin familial neonatal convulsions, generalized epilepsy with febrile seizures plus and autosomal dominant nocturnal frontal lobe epilepsy) and two of the progressive myoclonus epilepsies (Unverricht-Lundborg disease and Lafora disease) have been identified. The underlying genetic defects for common idiopathic generalized syndromes, such as absence epilepsies and juvenile myoclonic epilepsy, have not yet been precisely determined. Acquired epileptogenesis can be an acute or chronic process. The role of acute epileptogenesis in the final clinical expression of human epilepsy is unclear but needs further investigation. Among the chronic acquired partial epilepsies, temporal lobe epilepsy (TLE) due to hippocampal sclerosis has been extensively studied. Two hypotheses of epileptogenesis, involving structural reorganization, have been proposed – mossy fiber sprouting and dormant basket cell. Altered neurotransmitter expression, including increased activation of glutamate receptors and decreased GABA-mediated inhibition, is also important. Kindling and secondary epileptogenesis may play a role in some patients with intractable TLE. An autoimmune mechanism has been implicated in Rasmussen's encephalitis. Generalized absence epilepsy appears to result from a perturbation of the thalamocortical circuit. Clinical differences between seizures in neonates and infants, and those occurring in adults are likely related to maturational differences in cellular and molecular mechanisms. In the future, new therapies to prevent epileptogenesis (antiepileptogenic drugs) may be developed based on improved knowledge of the basic mechanisms of epilepsy.

THE term epileptogenesis refers to the transformation of the brain to a long-lasting state in which recurrent, spontaneous seizures occur[1]. It may involve a focal area of the brain (partial epilepsy) or the entire brain simultaneously (generalized epilepsy). Epileptogenesis must be distinguished from *seizure expression*, which is concerned with processes that trigger and generate seizures, because seizures can arise in nonepileptic brain exposed to acute insults.

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A knowledge of the mechanisms underlying epileptogenesis has considerable clinical relevance. The antiepileptic drugs (AED) currently used to treat patients with epilepsy affect seizure expression but a better approach in the future would be to develop agents that prevent epileptogenesis (anti-epileptogenic drugs). Experimental studies have shown that the two are not necessarily identical[1].

Epileptogenesis can occur in various ways. In general, these processes can be divided into genetic and acquired mechanisms. Acquired mechanisms can be acute or chronic. It is not yet clear which mechanisms are necessary or sufficient for the occurrence of seizures, but a number of recent studies have provided new insights into the genesis of epilepsy.

Genetic mechanisms

In the last decade, remarkable progress has been made in understanding the genetics of epilepsy. Over 13 genes associated with human epilepsy have been identified so far and at least 33 single gene mutations in mice have been linked to an epileptic phenotype. The list continues to grow in number and diversity. In the past, it had been assumed that generalized rather than partial epilepsies, and idiopathic rather than symptomatic epilepsies had a genetic basis. However, several genetic partial epilepsies have been recently identified and it now appears that some symptomatic epilepsies may have a genetic component[2]. Genetic advances in epilepsy are covered in detail in a separate review in this issue and therefore only a few syndromes in which the potential epileptogenic mechanisms have, at least in part, been understood are discussed in this article.

The mutant genes for three idiopathic epileptic syndromes – benign familial neonatal convulsion (BFNC), generalized epilepsy with febrile seizures plus (GEFS+) and autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) – are known. The common theme is that all three are due to mutations in genes coding for voltage-gated or ligand-gated channels, suggesting that these epilepsies are channelopathies. Further, it now appears that epilepsy, in general, may be regarded as part of a family of paroxysmal disorders such as episodic ataxia, periodic paralysis, familial hemiplegic migraine and cardiac arrythmias, all of which are also associated with mutant genes encoding a component of an ion channel[3].

BFNC is inherited in an autosomal dominant manner. Patients develop clonic or apneic seizures on the second or third day of life. Seizures typically stop within a week although they may recur in life in 10–15% of patients. By linkage analysis, loci were initially found on chromosome 20q13.3 and later on chromosome 8q24 in different families[4,5]. Subsequently mutations were identified in genes for previously unknown potassium (K) channels[6,7], which were named *KCNQ2* and *KCNQ3*. A similar gene expressed in the heart and mutated in the long QT syndrome[8] had been previously termed *KCNQ1*. The KCNQ potassium channels, which are identical to the M channels known to physiologists since 20 years[9] are different from the K channels that are involved in repolarizing action potentials. They are activated by small depolarization and because of their slow rates of opening and closing, allow firing of single action potentials but oppose sustained depolarization and repetitive firing. Mutations that cause BFNC are associated with loss of function of KCNQ channels leading to a decrease in the size of the potassium current. It has been suggested that even a moderate reduction (20–25%) of function may be associated with epilepsy[10]. It is not clear why a fixed genetic defect is associated with seizures only during the neonatal period but this may be related to developmental regulation of potassium channel expression.

GEFS+ is characterized by the occurrence of seizures with fever beyond the age of 6 years or afebrile generalized tonic-clonic, absence, myoclonic or atonic seizures in addition to febrile seizures[11]. The families described so far have followed an autosomal dominant pattern although it is believed that GEFS+ may more often follow complex inheritance. Two loci have been described, GEFS1 on chromosome 19q (ref. 12) and GEFS2 on chromosome 2q (ref. 13). A point mutation in the gene coding for the β-subunit of a voltage-gated sodium channel (*SCN1B*) was identified for GEFS1 in 1998 (ref. 12) and more recently, another mutation was found in the α1 sodium channel subunit (*SCNA1A*) gene[14]. *In vitro* functional studies have been performed in *Xenopus* oocytes expressing the mutant subunit. These suggest that the mutation results in defective inactivation of the sodium channel, which could lead to failure to limit the sustained repetitive firing of a depolarized neuron and be expressed as seizures[2].

ADNFLE begins clinically in childhood and patients have brief, nocturnal seizures with motor features. Two mutations have been identified in a ligand-gated channel – the α4 subunit of the neuronal nicotinic acetylcholine receptor (CHRNA4) on chromosome 20q13.2 (refs 15, 16). A second locus has been mapped to chromosome 15q24 which also contains subunits of nicotinic cholinergic receptors[17]. Recently the first mutation in a sporadic patient was found[18]. Mutations at the chromosome 20 locus results in decreased Ca^{++} flux through the receptor which may lead to a reduction in the amount of GABA release from presynaptic terminals and trigger a seizure by synaptic disinhibition[19].

The common idiopathic generalized epileptic syndromes such as absence epilepsies and juvenile myoclonic epilepsy have complex inheritance patterns. Several loci have been identified, but the underlying genetic defects remain to be determined. In general, the data support the presence of genetic heterogenicity.

In some of the symptomatic epilepsies, such as progressive myoclonus epilepsy (PME), seizures occur as part of a neurodegenerative disease. PME is a group of rare single-gene epilepsies characterized by myoclonus, generalized tonic-clonic seizures and progressive neurological dysfunction mainly in the form of dementia and ataxia[20]. Among the five major causes of PME, the gene and gene product have been identified in two diseases – Unverricht-Lundborg Disease (ULD) and Lafora Disease (LD). ULD is an autosomal recessive disorder with clinical onset between the ages of 6 and 16 years. It is characterized by prominent myoclonus, generalized tonic-clonic seizures, ataxia and mild dementia. The gene has been localized to chromosome 21q22.3 and encodes the protein *cystatinB* (ref. 21). Cystatin B is a member of the cysteine protease inhibitor family and its normal function is to inactivate proteins leaking from lysosomes. The most common mutation in ULD is an unstable dodecamer repeat expansion[22], which may cause loss of function and result in neuronal degeneration with epilepsy as a secondary effect[23]. A cystatin B knockout mouse model of ULD has been described but it has a phenotype that is different from that seen in humans[24]. These mice have ataxia and nocturnal myoclonus only with no daytime myoclonus or generalized tonic clonic seizures. Pathologically, there is pronounced apoptotic death of cerebellar granule cells, suggesting that loss of cystatin B function leads to a progressive increase in programmed cell death. The difference between the mouse and human phenotypes may be related to variation in the developmental expression of the gene as well as the presence of other genes that may respond to the loss of cystatin B by compensatory alterations. LD is first seen between the ages of 10–18 years and in contrast to ULD, is characterized by rapidly progressive, severe dementia and a fatal outcome in ~ 10 years after onset[25]. Pathologically PAS positive, diastase-resistant inclusions – Lafora bodies – are seen in a number of tissues and organs. LD is also inherited in an autosomal recessive manner and the gene has been localized to chromosome 6q24 (ref. 26). It encodes a protein tyrosine phosphatase that has been termed laforin[27]. Laforin is involved in glycogen metabolism, and regulation of ionic channels and synaptic transmission but the mechanism by which a deficit in laforin results in epilepsy is not clear. Not all patients with LD have this mutation, indicating the presence of

genetic heterogenicity. Some interfamily variations in the clinical features of LD have also been described[28] but no specific genotype–phenotype correlations have been performed so far.

Acquired mechanisms

Most studies on acquired epileptogenesis have focused on chronic mechanisms but in the last decade it has been recognized that epileptogenesis can also be an acute process. Acute epileptogenesis develops within minutes to hours and can, to some extent, be reversible whereas chronic epileptogenesis takes weeks, months or years to develop and is usually irreversible[1].

Acute

A number of *in vivo* and *in vitro* animal models of acute epileptogenesis have been described. The results suggest that common epileptogenic pathways for acute development of interictal epileptiform bursts and electrographic seizures are found in the hippocampus, neocortex and amygdala regardless of the nature of the inducing agent – stimulus trains, convulsant drugs or convulsant ion concentrations[1]. *In vitro* models of status epilepticus have shown that epileptogenesis can be progressive even after the epileptogenic agent or stimulus is withdrawn and that cell loss can occur acutely, possibly as a result of seizures. In both animal and human studies, it has been found that AED may lose effectiveness over the course of a prolonged episode of status, suggesting that this transition may be a form of epileptogenesis specific to status epilepticus[1]. The proposed molecular mechanisms of acute epileptogenesis include NMDA receptor activation and calcium influx with secondary changes in the form of increased AMPA and NMDA synaptic transmission, acute decrease in GABAergic inhibitory synaptic transmission, and an increase in net excitatory effects, leading to increases in ectopic action potentials or depolarizing potentials. Nonsynaptic mechanisms such as changes in coupling through gap junctions[29], iron-mediated changes in Ca^{++} oscillations or glutamate release[30] and generation of oxygen-free radicals[31] may also be important. It appears that acute neuronal loss alone is neither necessary for the generation of acute epileptiform bursts *in vitro*[32] nor sufficient for the expression of spontaneous seizures *in vivo*[33]. It is not clear if acute epileptogenesis plays a significant role in the final clinical expression of human epilepsy, but if it does, the time frame for antiepileptogenic therapy in such cases may be very narrow. On the other hand, it may allow for short-term therapy over a period of days or weeks rather than the traditional prolonged treatment for most forms of epilepsy. Further investigation of acute epileptogenesis is therefore warranted.

Chronic

Partial epilepsy. It has been known for a long time that brain injury from trauma, stroke, infection, etc. results in epilepsy but the molecular and cellular mechanisms underlying the epileptogenesis are not well understood. Although the mechanisms may be different in different patients, certain features are common to most forms of acquired partial epilepsy: a latent period of weeks to years between the injury and emergence of epilepsy, the high incidence of anatomic changes with neuronal loss, involvement of synaptically connected but anatomically distant sites and its long duration or even permanence[34]. Of the partial epilepsies, temporal lobe epilepsy (TLE) due to hippocampal sclerosis is very common and has been extensively studied with respect to mechanisms of epileptogenesis in the last decade. It is therefore discussed in detail below.

Normal hippocampal anatomy and structural reorganisation in epilepsy. The hippocampus consists of the Cornu Ammonis subfields CA1 through CA4 and the dentate gyrus. The primary neurons of the Cornu Ammonis are the pyramidal cells while those of the dentate gyrus are granule cells. The axons of granule cells are called mossy fibers and these are normally not seen in the inner molecular (supragranular) layer where the dendrites of granule cells are present. The hippocampal circuitry consists of a trisynaptic excitatory pathway – from the entorhinal cortex to the dentate granule cells, which project to the CA3 pyramidal neurons via mossy fibers, and from there to the CA1 region through Schaffer collaterals[35]. There are local circuits in each region with excitatory and inhibitory interneurones. The CA3 region is most prone to epileptiform activity[36], partly because of excitatory connections with neighbouring cells. On the other hand, it is difficult to induce seizures in granule cells, in part due to the lack of excitatory connections with neighbouring granule cells and the presence of strong polysynaptic inhibitory synapses on granule cells[2]. Normally, dentate granule cells limit seizure propagation through the hippocampal network[37,38]. However, structural reorganization with alteration of the synaptic circuitry may transform the granule cells into an epileptogenic population and promote seizure initiation and/or propagation. Two hypotheses of enhanced epileptogenesis in the granule cells have been proposed in the last decade.

The mossy fiber sprouting (MFS) hypothesis refers to the synaptic reorganization of the mossy fiber axons of the granule cells so that they project into the inner molecular layer of the dentate gyrus and make excitatory contact with granule cell dendrites, resulting in a recurrent excitatory circuit which eventually leads to seizures[39,40]. The explanation offered for the fact that

spontaneous seizures occur only intermittently, despite a fixed structural change, is that intact synaptic inhibition normally suppresses the effect of the excitatory circuit[2]. Seizures occur only when inhibition is transiently decreased. There is pathological and physiological evidence in support of this hypothesis. Mossy fibers and their anatomical rearrangements can be readily identified by the Timm stain because of their high content of zinc. From whole-cell patch-clamp recordings of dentate granule cells in hippocampal slices isolated from kainate-treated animals, it has been confirmed that at least some of the MFS forms functional recurrent excitatory circuits[33].

The precise stimulus that causes MFS is not known. Because MFS is typically seen in a sclerotic hippocampus with extensive neuronal loss, it has generally been believed that death of susceptible neurons such as the mossy cells, which normally project to the inner molecular layer (axons of mossy cells must not be confused with mossy fibers, which are the axons of granule cells), is the initiating stimulus. Axons of the surviving neurons then fill up the vacated synapses[2]. Further, seizures themselves may kill the susceptible neurons and set up a vicious cycle – seizures cause neuronal death, which leads to MFS, which in turn leads to more seizures. Many studies[41–43] have shown that seizure activity alone, if sufficiently prolonged or vigorous such as status epilepticus, causes death of vulnerable neurons. A recent study suggests that brief, repeated seizures also can cause neuronal death[44]. An interesting form of neuronal plasticity related to granule cells has also been identified recently. Unlike most neurons in the central nervous system, neurogenesis of granule cells normally persists into adulthood. Seizures not only cause granule cell death but also increase neurogenesis[45]. However, MFS appears to arise from mature granule cell death rather than newborn cells[46]. Apart from neuronal death, MFS may result from seizures triggering a cascade of gene expression including immediate early genes such as *c-fos* and *c-jun*[47], and genes coding neurotrophic factors[48] and axonal growth-associated proteins such as *GAP-43* (ref. 49).

Despite evidence of its existence, the role of MFS and recurrent excitatory circuits in generating seizures have been questioned by some authors. Sprouting occurs after hippocampal ischemia but is rarely epileptogenic[34]. In animal studies, sprouting can occur without development of spontaneous seizures[50]. Conversely, seizures can occur with severe to complete inhibition of sprouting[51]. Mossy fiber synapses with GABAergic neurons have also been found[52], raising the possibility that MFS may promote inhibition of granule cells through a disynaptic pathway. Indeed, it has even been suggested that MFS predominantly targets inhibitory cells and restores normal inhibitory responses, instead of setting up an excitatory circuit[53].

An alternative hypothesis focuses on the role of decreased inhibition. Recurrent, intense seizures are known to cause loss of GABA-mediated inhibition of granule cells. However, from detailed immunohistochemical studies of animal and human sclerotic hippocampus, Sloviter[54] found that certain inhibitory cells (basket cells) are actually more resistant to seizure-induced death, in contrast to the excitatory mossy cells and somatostatin-containing neurons in the dentate hilus, which are highly sensitive. The paradox of functional loss of inhibition with selective preservation of inhibitory (basket) cells was resolved by the dormant basket cell hypothesis (DBC) proposed by Sloviter[55]. According to this hypothesis, seizure-induced death of mossy cells results in lack of the normal excitatory input to the basket cells which, although preserved, cannot provide feedback inhibition to granule cells and remain in a dormant state. Because mossy cells are injured at the time of the original seizures and disappear within a few hours or days, it is difficult to explain the usual long latency seen between the initial injury and the development of epilepsy by a simple 'loss of inhibition'[34]. However, it has been suggested that the partial loss of inhibition combined with excitatory synaptic input due to otherwise normal stimuli may lead to excessive firing of granule cells, progressive cell death and emergence of epilepsy many years later[56].

Other authors[57,58] have attempted to test the DBC hypothesis and, in general, the results are not supportive. Hilar mossy cells innervate some interneurons within the same lamina but their longitudinal projections appear to innervate granule cells and not inhibitory interneurons[59]. Also basket cells appear to be active rather than dormant in some models of TLE[57,58]. Despite these problems, the complexity of the hippocampal circuitry makes it difficult to fully test or reject this theory.

Cellular mechanisms and neurotransmitters. The intracellular correlate of an interictal spike on the EEG is the synchronized occurrence of a paroxysmal depolarizing shift (PDS) in a group of neurons[60]. Johnston and Brown[61] showed that PDS is a network-driven phenomenon resulting from an imbalance between excitation and inhibition. The synchronizing potential is a giant excitatory post-synaptic potential (EPSP), which is mediated by glutamate. PDS is mediated largely by the NMDA and AMPA types of ionotropic glutamate receptors, although metabotropic receptors also play a role by modulating the frequency of PDS discharges[62]. In hippocampal slices and cultures, NMDA receptor activation by lowering extracellular magnesium, which normally blocks the receptor, leads to seizures[63]. In normal brains and hippocampal slices, NMDA receptors do not participate in low frequency synaptic transmission but in epileptic brains they may be recruited into

synaptic transmission[64]. This increased activation of NMDA receptors may be due to expression of novel receptors[65] or altered regulation by a calmodulin-dependent phosphorylase, calcineurin[66].

In addition to excitatory connections, reduction of inhibition is necessary for synchronization of bursts. GABA-mediated inhibition normally holds the membrane potential below the action potential threshold and prevents recruitment of bursts. It also prevents synchronization of intrinsic burst discharges in pyramidal neurons by decreasing the connectivity of their divergent, polysynaptic excitatory pathways[67]. In epileptic brains, decreased inhibition increases the probability of firing action potentials in response to an EPSP and allows synchronization of burst discharges. Recently, it has been speculated that decreased inhibition in TLE may be due to alteration of the molecular structure of $GABA_A$ receptors in the hippocampus. As discussed above, in epileptic brains, mossy fibers aberrantly innervate granule cells and release zinc. In normal rats, the $GABA_A$ receptors on dentate granule cells are not sensitive to zinc but, in epileptic rats and humans, the subunit composition of the $GABA_A$ receptor, which determines its properties, may be altered by zinc and lead to changes in function[68].

Other mechanisms of partial epileptogenesis. Although an autoimmune mechanism for epilepsy was suspected more than 50 years ago[69], only recently has evidence emerged to support this hypothesis in a rare form of epilepsy – Rasmussen's encephalitis (RE). RE is a progressive degenerative disease affecting children. Patients have seizures that are typically refractory to AED, and progressive hemiparesis with dementia. Recent data indicate that RE may be due to an immune response directed against the GluR3 subunit of the AMPA glutamate receptor[70]. Plasma exchange involving removal of circulating IgG and IgM is associated with clinical improvement and reduction in GluR3 antibody titers[71]. Anti-GluR3 activates AMPA receptors but the mechanism of its cytotoxic effects on neurons appears to be complement-mediated rather than excitotoxic[72]. Access of the antibody to neuronal epitopes in the CNS is necessary to trigger this complement-mediated damage[73]. Cell-mediated immunity may also be altered in RE. Subpopulations of T lymphocytes appear to be overrepresented in the RE brain tissue as compared to the T Lymphcytes in the blood[74]. This subset may be part of a selective attack on one or more antigens that remain to be identified[2].

Generalized epilepsy. While partial epilepsy has been extensively studied for the last 30 years, advances in the mechanisms underlying generalized epilepsy, especially absence epilepsy have been made only in the last decade[75]. From both animal and human data, it appears that the 3 Hz generalized spike and wave pattern results from a perturbation of a physiological, higher frequency thalamocortical oscillatory rhythm. Thalamocortical rhythms form the substrate of the neurobiology of sleep[76], and the mechanisms underlying sleep spindles and the generalized spike and wave EEG pattern may be related. Because sleep spindles originate in the thalamus, it appears to be the pacemaker in absence seizures but the participation of the cortex is essential, and an intact thalamocortical loop is necessary for the full expression of absence seizures. In order to understand the proposed pathogenesis of absence seizures, the normal thalamocortical circuit must first be reviewed.

Thalamocortical oscillations are generated by synaptic interplay of three structures – nucleus reticularis thalami (nRT), thalamocortical neurons (TCN) and cortical pyramidal neurons. nRT neurons receive excitatory collateral projections from the TCN and from the cortical cells projecting to the thalamus. nRT neurons send *inhibitory* projections to the TCN but not to the cortex. Thus, a reciprocal excitatory-inhibitory connectivity exists within the thalamus. Both nRT neurons and TCN have an intrinsic ability to fire in bursts when their cell membrane is *hyper*polarized. This process is dependent on extracellular Ca^{++} and the Ca channels responsible are of the T-type[77,78]. Due to high level of T-channel expression, thalamic neurons fire action potentials in high-frequency, short duration bursts after membrane hyperpolarization, in contrast to the regular firing pattern in the absence of hyperpolarization. Burst firing in the nRT cells results in high-frequency GABA dependent inhibitory post-synaptic potentials (IPSPs) in the TCN. These IPSPs summate and produce large potentials mediated by both $GABA_A$ and $GABA_B$ receptors[79]. The hyperpolarization associated with the IPSPs, particularly that mediated by $GABA_B$, acts as a priming mechanism for the T-channels in the TCN so that a rebound Ca-dependent burst is evoked. The rebound burst in turn excites nRT neurons. If the resting membrane potential of the nRT neurons is sufficiently hyperpolarized, then the incoming EPSPs, modulated by glutamate, evoke Ca-dependent bursts leading to a reentrant activation of the circuit[79]. Thus, specific resting conditions are necessary for recurrent activation – TCN must be sufficiently depolarized at rest so that nRT-evoked IPSPs result in a rebound response, and nRT cells must be sufficiently hyperpolarized at rest so that incoming EPSPs can directly evoke a burst response. TCN transfer bursts to the cortex where they induce EPSPs in pyramidal neurons, thereby generating EEG spindle waves[80]. The overall set point of thalamic and cortical excitability, and the ultimate expression of absence seizures, is also modulated by ascending cholinergic pathways projecting to the thalamus, and by noradrenergic and dopaminergic neurons projecting to the cortex (layers V and I)[75]. In addition, presynaptic $GABA_B$ recep-

tors may regulate thalamocortical rhythmicity by modulation of GABA release within the circuit.

The main factor that controls the timing of the circuit oscillation is the duration of IPSPs in TCN[81], which depends on the type of GABA component that is more prominent. If the IPSP is mainly mediated by $GABA_A$ as seen in ferret visual thalamic slices[82], the resultant network frequency is ~ 8 Hz. On the other hand if the IPSP is prodominantly $GABA_B$ mediated, as in rat somatosensory thalamus slices[79], 3 Hz oscillations are seen. The importance of $GABA_B$ receptors in absence seizures is demonstrated in genetic animal models by the fact that thalamic injections of selective $GABA_B$ agonists result in increased incidence of spike-wave discharges, whereas injections of $GABA_B$ antagonists decrease the discharges in a dose-dependent manner[83,84]. Linkage between spike-wave discharges and T-channels is provided by the fact that ethosuximide, a potent anti-absence drug, acts by blocking the T-type Ca current[85]. However, in humans what precise aberration of the thalamocortical circuit occurs in absence seizures is not known. Other unanswered questions of absence seizures include: what stops the spike-wave discharge? Why does the clinical presentation of absence seizures vary within different syndromes? What is the cause of generalized tonic-clonic seizures later in life in 45–50% of patients?

Kindling and secondary epileptogenesis: Current status

Gowers'[86] famous statement 'seizures beget seizures', made a century ago, suggested that epilepsy is a progressive disorder and initiated a controversy that persists to this day. It is now clear that in the vast majority of patients treated with AED, epilepsy is not a progressive disorder. However, the phenomena of secondary epileptogenesis, mirror focus and kindling suggest that in experimental animals, and possibly in some humans with intractable epilepsy, the disorder may be progressive and repeated seizures may promote additional seizures[87].

A primary epileptogenic area has a macroscopic abnormality and can generate seizures independent of the presence of surrounding or remote epileptogenic areas[88]. A secondary epileptogenic area becomes epileptogenic because of the influence of epileptogenic activity in a primary epileptogenic area, which is separated from it by at least one synapse[89]. The cortex in the secondary epileptogenic area is normal other than the changes induced by the primary epileptogenic area. A mirror focus is a type of secondary epileptogenesis in which the secondary epileptogenic zone is located in a contralateral homotopic area with regard to the primary epileptogenic zone[90]. Secondary epileptogenesis likely occurs due to kindling, a phenomenon originally described by Goddard[91], that is associated with a permanent and progressive epileptic condition through repeated, brief stimuli that initially do not evoke epileptic discharges.

The kindling model of epilepsy has firmly established that, at least in animals, seizures can beget seizures. Pathways in the limbic system and temporal lobe are particularly susceptible to kindling[91] and recent studies have shown that many of the cellular and functional changes noted with kindling are remarkably similar to those seen in humans with intractable TLE, such as progressive mossy fiber sprouting, neuronal loss and memory disturbances[87]. TLE often evolves to a progressive disorder after an inciting event or pathological insult, such as febrile seizures, that appears to be self-limited[92]. It has been suggested that, although kindling may not play a role in the pathological process immediately following the initial injury, the cumulative functional effects of repeated seizures may account for some features of the epilepsy that develop after the initial inciting event and for progression to intractability. However, the occurrence of kindling has not been directly demonstrated in human hippocampal circuitry and TLE due to the inability to serially study functional and cellular alterations. Even in animals, kindling develops and evolves more slowly in phylogenetically advanced species such as primates. Therefore, it is possible that kindling to the point of spontaneous seizures does not occur in humans. Nevertheless, it is possible that the cumulative effects of repeated seizures may contribute to some features of TLE even if kindling-like processes alone are not sufficient to induce spontaneous seizures[87]. Genetic factors may determine the susceptibility to destructive effects of repeated seizures and the risk for progression, as suggested by recent studies demonstrating the existence of fast-kindling and slow-kindling strains of rats[93], and may eventually provide markers for identifying subsets of patients with similar vulnerabilities. Overall, kindling currently is best regarded as a model of seizure-induced circuit plasticity that may play a variable role in different epileptic syndromes or may influence a subset of patients with TLE destined for progression and intractability[87].

Secondary epileptogenesis progresses through three stages as described by Morrell and Tsuru[94,95]. In the dependent phase, epileptiform discharges in the secondary epileptogenic zone are always time-locked to those in the primary epileptogenic zone and disconnection of the secondary from the primary zone leads to immediate cessation of all discharges in the secondary zone. In the intermediate phase, the primary and secondary zones generate independent (i.e. not time-locked) spikes (interictal intermediate phase) or independent seizures (ictal intermediate phase) but all of the epileptic manifestations disappear after a variable interval when the

secondary zone is disconnected from the primary zone. Finally, in the independent phase, the secondary zone generates independent discharges and seizures that can no longer be abolished by disconnection from the primary zone. It is not clear if independent secondary epileptogenesis occurs in humans but this has obvious therapeutic implications. Epilepsy surgery is a widely available option and a successful outcome depends on restriction of the focus to a primary resectable area. If independent secondary epileptogenesis does occur, early surgery would clearly be indicated, before the development of an independent mirror focus.

There is some clinical evidence to support the occurrence of acute secondary epileptogenesis. During a seizure, cortical areas that are otherwise normal may generate brief (seconds to minutes) epileptic discharges. These may sometimes persist for a few minutes after the primary epileptogenic area has stopped firing, giving rise to clinical phenomena such as late 'paradoxical version' (towards the side of the primary epileptogenic zone)[96]. During status epilepticus, seizures may sometimes arise from secondary epileptogenic zones[88]. There is also strong clinical evidence for both interictal and ictal chronic intermediate secondary epileptogenesis[97–99]. However, it is difficult to provide conclusive proof of chronic independent secondary epileptogenesis in humans. The only evidence supporting its occurrence has been provided by Morrell[100] in 8 patients with tumours, all of whom developed seizures with a completely different clinical semiology after resection of the primary focus. Lüders[101] has argued that this evidence is inconclusive since it is often difficult to lateralize or localize the seizure focus based on clinical semiology alone. He also is not convinced that the only example of an EEG-documented seizure provided by Morrell truly arises from the contralateral side. Therefore, at present we cannot conclude with certainty that independent secondary epileptogenesis occurs in humans. Even if it does occur, the difficulty in finding well-documented cases suggests that it must be a very rare phenomenon[88].

Developmental aspects

There are several clinical differences between seizures in the immature brain as compared to those in adult brain. Neonates and infants are more susceptible to seizures than adults[101], and have different seizure characteristics[102,103] and responses to AED. Also, certain types of seizures in early life, such as febrile seizures, are associated with increased likelihood of chronic epilepsy later in life and may themselves contribute to epileptogenesis[104,105]. These clinical variations are likely related to maturational differences in the cellular and molecular mechanisms of epilepsy. A knowledge of these mechanisms is helpful in understanding the age-dependence of childhood epilepsies and optimizing age-appropriate treatments.

Several factors may be responsible for increased excitability of the immature brain. From animal studies, it appears that the early postnatal period represents a developmental window with ongoing synaptogenesis and increased neuronal plasticity as compared to adults[106]. There is an overshoot of synaptic density before it is pruned to adult levels[107]. This is associated with a parallel over expression of glutamate receptors, which is required for these normal developmental processes. NMDA, AMPA and kainate receptors increase over the first few weeks of life, resulting in certain brain regions being more susceptible to the epileptogenic and excitotoxic effects of glutamate-receptor-agonists during this period[108]. The expression of the NR2B NMDA receptor subunit, which is associated with increased duration of NMDA receptor-mediated excitation and increased Ca^{++} influx, is higher during the postnatal period[109]. Due to the relative underexpression of the GluR2(B) subunit, a larger proportion of AMPA and kainate receptors show permeability to Ca^{++} in the immature brain as compared with the mature brain[110]. The development of metabotropic glutamate receptors also favours a hyperexcitable state since the activity of post-synaptic receptors that promote increased intrinsic neuronal excitability predominates over that of receptors that presynaptically inhibit glutamate release[105]. Finally, a relatively lower activity of some glutamate transporters could contribute to a greater susceptibility to seizures[111].

The increased excitability could also be related to developmental differences in inhibitory mechanisms. In the first postnatal week, $GABA_A$ receptor activation causes membrane depolarization rather than hyperpolarization as seen in the mature brain, due to maturational changes in the transmembrane chloride ion gradient[112]. Inhibitory hyperpolarizing potential gradually appears over the first 3 weeks, associated with the induction of expression of the neuronal K^+/Cl^- cotransporter KCC2, which extrudes Cl^- from cells[113]. With regard to $GABA_B$ receptors, activation of the presynaptic receptors, which decrease GABA release, occurs earlier in development than activation of polysynaptic receptors that mediate long-lasting hyperpolarization[114]. The net result is increased excitability. As noted above, $GABA_B$ receptor activation is important in the generation of absence seizures. The age-dependence of absence seizures has been related to a mismatch between the rates of maturation of $GABA_B$-mediated and glutamate receptor-mediated transmission in thalamocortical circuits, with the latter lagging behind the former before adulthood[75].

Maturational differences also occur in other neuromodulatory substances. For instance, corticotropin-releasing hormone (CRH) is highly epileptogenic and

particularly important in triggering seizures by fever or hypoxia in the immature brain[115]. CRH receptor expression has been estimated to be twice the adult level in immature rat amygdala in the second postnatal week[116].

The developmental patterns of voltage-gated ion channels promote increased intrinsic membrane excitability during early postnatal development because this is necessary for normal development[117]. Changes in presynaptic Ca channels that mediate neurotransmitter release may be particularly important in determining seizure susceptibility[105]. Intracellular Ca homeostasis is also developmentally regulated. The calcium-binding protein calbindin D28K is not expressed in immature hippocampal granule cells[118]. The major neuronal Na^+/K^+ ATPase also is less abundant in the immature brain so that a moderate increase in neuronal activity could cause extracellular K^+ to rise to epileptogenic levels[119].

The mature nervous system may also be far more plastic than previously thought. Recent data presented at the 2001 annual meeting of the American Academy of Neurology by Lowenstein suggest that a number of processes that occur during development such as neuronal differentiation, axonal guidance and branching continue to occur in the mature animal. These processes appear to be dependent on a number of molecules such as Notch, Mash, Netrin, Semphorins, etc. which are now known to be expressed in the fully developed, normal, adult nervous system as well. As discussed above, chronic acquired epileptogenesis, e.g. TLE, is associated with alterations in synaptic and axonal reorganization. It has been speculated that these changes may be influenced by altered expression of these molecules. Further, several potential strategies in the future might use these developmental mechanisms to restructure the neural networks of a seizure focus in such a way that the normal balance of excitation and inhibition is restored. The phenotype of existing cells could be modified, axonal reorganization could be blocked or encouraged, neurotransmitter or receptor expression could be altered, the cell fate of endogenous or exogenously induced stem cells could be modified or local network properties could be modulated by introducing functional biological or electronic interfaces.

Conclusions

Although considerable advances have been made in understanding the basic mechanisms of genetic and acquired epilepsies in the last decade, several challenges lie ahead. As molecular genetic techniques continue to evolve, several new genes and genetic syndromes are likely to be discovered. However, the relationship between the genetic defect and functional consequences need to be better understood. New methods such as cDNA microarray technology may help in studying gene expression. Study of the genetics of developmental anomalies such as periventricular heterotopias may shed light on mechanisms of epileptogenesis in neurologically normal individuals. Also, the genes involved in the more common forms of epilepsy, remain to be clearly identified. The formation of recurrent excitatory pathways appears to be an important theme in acquired epilepsies but the precise extent to which these contribute to epileptogenesis *per se* is not clear. Other unanswered questions include: If the periodic occurrence of seizures is related to brief reduction in synaptic inhibition, what is the trigger? Does the hyperexcitable network subserve recovery of function after brain injury and, if so, to what extent? Will inhibiting formation of this network therefore have some harmful effects? What are the molecular cues underlying formation of this network?[2] We have some preliminary answers based on studies of normal development but these need to be clarified further. The precise role of abnormal neuron–glia interaction and of neurotransmitters including substances such as adenosine and nitric oxide, which have been recently implicated in epilepsy, also needs to be defined. Newer imaging techniques such as PET with a wide array of receptor ligands, magnetic resonance spectroscopy, diffusion-weighted MRI and functional MRI will provide a better understanding of the biochemical functions of chronic epileptic brains *in vivo*.

Ultimately, the greatest challenge will be to find new treatments based on improved knowledge of the basic mechanisms of the epilepsies. Again, multiple approaches are likely to be used. Conventional AED are being studied for their antiepileptogenic potential and some preliminary data suggest that vigabatrin, topiramate, and zonisamide may be helpful. Vagal nerve stimulation and the ketogenic diet have also been shown to have antiepileptogenic effects in some studies. New drugs are likely to be developed, based on an understanding of the genetic mechanisms of epilepsy. One such drug, retigabine, which is a potent KCNQ channel opener, has already shown some promise and is currently undergoing trials. Ca channel blockers and glutamate-aspartate antagonists may help in preventing epileptic cell damage. Somatic or germline gene therapy may be particularly useful for the fatal forms of genetic epilepsies, such as Lafora disease, in the future. With continued cooperation among clinicians, geneticists and basic scientists, the next decade is likely to be even more exciting than the previous one and we may eventually achieve the goal of curing epilepsy, or preferably, preventing the process of epileptogenesis from occurring in the first place.

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