SECTION B ■ EPILEPTOGENESIS, GENETICS, AND EPILEPSY SUBSTRATES

CHAPTER 3 ■ EXPERIMENTAL MODELS OF SEIZURES AND MECHANISMS OF EPILEPTOGENESIS

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ANIMAL MODELS OF SEIZURES AND EPILEPSY: WHAT IS THE QUESTION?

An editorial (1) began to summarize one of the key issues in experimental models of seizures and epilepsy: how close does your model need to be to the true human condition in order to reach valid translational conclusions? In other words, is the best model for a cat actually a cat, preferably the same cat (2), or will a dog do because it also has fur? To some extent the differences between the cat and dog are irrelevant, as our understanding of the mechanisms of brain processes from development to learning and memory in healthy and diseased states are still in their infancy. The first step, undoubtedly, is to define the pertinent questions. For the pediatric epilepsies, this has been approached in a workshop "Models of Pediatric Epilepsies" sponsored by NIH/NINDS, the American Epilepsy Society and the International League against Epilepsy (3). Those questions were as follows: (i) what are the long-term consequences of seizures? Can these be modified? and (ii) what is the best anticonvulsant therapy? What is the best antiepileptogenic therapy? From these questions, the mechanisms of seizure initiation, prolongation, and termination can be addressed, and their sequelae defined. Further, the mechanisms underlying the development of spontaneous repetitive seizures (SRS) (epileptogenesis) and associated cognitive dysfunction can begin to be addressed. The mechanisms by which modifiers such as genetic background, developmental stage, and other insults (hypoxia, trauma) may also be differentiated. From this, the committee proposed a table listing general strategies for model development (Table 3.1). In brief, models should be clinically relevant, developmentally appropriate, and generalize to a human condition (i.e., have validity).

While entire volumes have been devoted to the subject of this chapter (4,5), we will review the literature involving only a subset of the issues that seem pertinent to a text on clinical epilepsy. Following a review of synaptic transmission mechanisms, we will focus on the methods for invoking status epilepticus (SE) (a "prolonged" single seizure) via chemoconvulsants; single, repetitive, or prolonged seizures via hypoxia, temperature, kindling, or chemoconvulsants; and seizures induced by trauma or genetic alterations. The process by which the initial insult (seizure, SE, or other) may lead to spontaneous SRs (epilepsy) has been the subject of intense study and multiple reviews have been put forth (6,7). Consensus regarding the relationship (cause or effect?) of sclerosis and network reorganization to this process has not been forthcoming. Overall, the field has significantly shifted from a descriptive to a mechanistic focus involving key receptors, enzymes, and genetic regulation.

TABLE 3.1

STRATEGIES FOR ANIMAL MODEL DEVELOPMENT

1. Address a clinical need for better therapies
2. Address a key question or testable hypothesis
3. Address age specificities of developmental epilepsies and exhibit age-specific manifestations
4. Address normal aspects of development as they relate to models of developmental epilepsies
5. Animal models of seizures and epilepsy should have EEG correlates; spontaneous seizures should be demonstrated in animal models of epilepsy
6. Investigate etiology and natural history of catastrophic/intractable epilepsies
7. Address role(s) of "multihit" mechanisms in epileptogenesis and epilepsies, that is, trauma plus seizure or environment/diet plus genetic susceptibility
8. Address long-term role of seizures and other aspects of epileptic encephalopathies
9. Address model validity to clinical situation by comparisons with pharmacologic response, seizures phenotypes, outcomes, genetics, and so on
10. Allow cross-pollination from related fields: ischemia, sleep, trauma, synaptic plasticity, cancer/cell-signaling, and so on

Modified from Stafstrom CE, Moshe SL, Swann JW, et al. Models of pediatric epilepsies: strategies and opportunities. *Epilepsia*. 2006;47:1407–1414.

GENERAL MECHANISMS OF TRANSMISSION AND NETWORKS

Seizures can be defined as paroxysms of abnormal, rhythmic, synchronized discharges in the brain. Communication in the nervous system is a combination of electrical and chemical signaling with a balance between excitation and inhibition in each, primarily mediated between neurons. Glia modulate both types of communication primarily on a local basis, but frequently with distant consequences. While neurons are largely polarized structures favoring directed communication (an input

end and an output end), this is not always the case and how this may change is clearly relevant to seizures. As electrical units, neurons depend on membrane-embedded protein ion channels to maintain their membrane in a polarized state in which, at rest, the inside of the neuron is electronegative compared to the outside. Each ion channel has its own relative ion selectivity and the net directional flux of ions (which depends on both the concentration of ions on either side of the membrane and the membrane polarity or voltage) determines whether this flux will move the neuronal membrane voltage toward, or away from, its resting state. Ionic channels transition between opened and closed states. This gating can be modulated by membrane voltage (voltage-gated channels [VGCs]) and/or the binding of external or internal chemical ligands.

Synaptic transmission is the process by which neurotransmitters (ligands) released from a neighboring neuron diffusively move toward another neuron and bind to receptors on that neuron. Ligand binding to a receptor can result in channel opening within the receptor or lead to the ligand-bound receptor interacting with a separate protein, often another channel, as in the case of G-protein-coupled receptors (GPCRs). Neurotransmitter release involves many tightly linked processes. Only specialized structures and regions are involved in neurotransmitter release. Initiation of release involves either local voltage-gated mediated polarization changes or second messenger systems activated by neurotransmitters themselves. Vesicles, membranous spheres filled with neurotransmitter by pumps within the vesicular membrane, then fuse with presynaptic membranes to release neurotransmitter into the synaptic cleft that separates the presynaptic neuron from the postsynaptic neuron. Less commonly, neurotransmitters may be directly pumped into the cleft. Neurotransmitters are either enzymatically degraded in the cleft or pumped out of the cleft by transporters into the presynaptic terminal, postsynaptic neuron, or surrounding glial support cells. From there it is either enzymatically broken down, recycled and shuttled across membranes, resynthesized or pumped backed into vesicles.

The resulting ionic flux(es) can have several simultaneous consequences. Some ions only affect membrane voltage while certain ions (e.g., calcium) also act as second messengers by activating calcium-dependent enzymes. These enzymes can then exert a cascading effect on ion channels and other enzymes, including those that influence membrane shape and scaffolds that hold and direct protein location (i.e., internal versus external, synaptic versus extrasynaptic), protein translation, protein degradation, and RNA transcription.

Neurons are three-dimensional structures with compartments (dendrite, axon, and soma) and subcompartments in each (e.g., main dendrite, branch, spine; axonal hillock, axon, branch, terminal), and the precise temporal and spatial regulation of neuronal function is mirrored by the segregation of unique, but often similar, ion channels and enzymes to distinct subcompartments. For instance, the molecular diversity of potassium channels, each coded by different genes and often many splice variants, reflects the unique functional needs or duties of each subcompartment where they may be selectively located and regulated. Neurons themselves are also segregated as inhibitory or excitatory, depending on the type of neurotransmitter(s) they may (predominantly) release. Each class of neuron may also express a unique complement of ion channel and receptor subtypes resulting in incredible diversity of neuronal function.

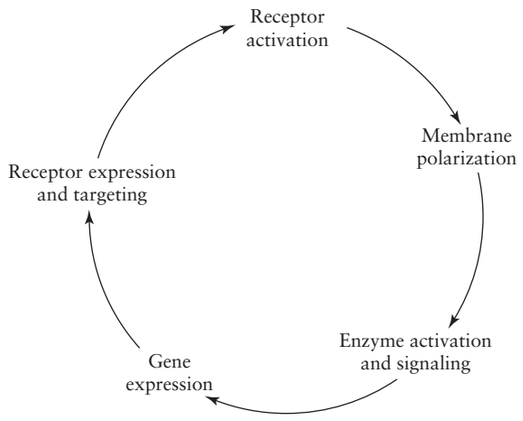

FIGURE 3.1 Proposed cascade of events following a seizure leading to any potential adverse sequelae (status epileptics, epileptogenesis, learning impairment, etc.).

The resulting cascade, beginning with receptor activation, followed by alterations in membrane polarization, potentially loops around to result in alterations of the properties of the initial trigger of receptor activation. Consideration of this simplistic mechanism is important. Such a loop likely underlies normal plasticity associated with processes like learning and memory, but perhaps becomes unstable with seizures and epileptogenesis, leading to aberrant plasticity that could result in both seizures and cognitive dysfunction (Fig. 3.1).

Glutamatergic Ion Channels

At the synaptic level, most excitatory amino acid transmission in the central nervous system (CNS) is mediated by the activation of families of glutamate-activated ligand-gated cation channels classified according to their preferred agonists: kainate, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), or *N*-methyl-D-aspartate (NMDA) (8). To date, nine subunit subtypes and related isoforms have been cloned with pharmacology in in vitro expression systems similar to the AMPA (GluR1-4) and kainate receptors (GluR5-7, KAR1-2) (9–11). Similarly, five subunit subtypes and related isoforms have been cloned with pharmacology in in vitro expression systems similar to NMDA receptors (NR1, NR2A-D) in vivo (12–16). Some subunit-specific interactions and their role in synaptic transmission have been shown (17–22). Metabotropic glutamate receptors (mGluRs) are GPCRs broadly divided into three classes (Groups I–III) (23). Epileptologists are becoming increasingly interested in ionotropic glutamate receptors as the anticonvulsants topiramate, felbamate, and talampanel likely interact with these receptors. In addition, Group I mGluR agonists or Group II mGluR antagonists are thought to have both anticonvulsant and antiepileptogenic potential (24). Since these modulatory receptors do not directly participate in fast excitatory synaptic transmission, it is hoped that targeting these receptors may be effective with fewer side effects compared to agents that directly modulate GluRs and NRs.

Calcium influx through NRs is thought to mediate the calcium-activated processes involved in long-term potentiation and depression (LTP and LTD) (25–29), neurite outgrowth (30), synaptogenesis (31), and cell death (32–34). LTP and

LTD are thought to be synaptic models of learning and memory (35). "Induction" of LTP/LTD is thought to take place when synaptically activated NRs allow calcium entry and accumulation in the neuron. In order for this to happen, the nearby region of dendrite must be sufficiently depolarized by synaptic activation of GluRs to alleviate the magnesium-dependent block of NRs. "Expression" of LTP/TD is thought to occur when calcium activates a cascade involving protein phosphorylation and dephosphorylation resulting in modifications in synaptic strength (36).

While other possible mechanisms exist (35,37–40), postsynaptic changes in GluR subunit numbers (40–42) or properties (42) are thought to underlie synaptic modification. This has resulted in postulated "subunit rules": (i) for AMPA-type GluRs, synaptic removal of GluR2 subunits drags along GluR1 and GluR3 which underlies LTD, (ii) GluR1 or GluR3 not associated with GluR2 ("homomers") act independently, (iii) insertion and/or modification of GluR1 underlies LTP (43). It is likely that the regulation of GluR subunits and measured properties are exquisitely intertwined (44). Knockout studies of GluR1 (45) and GluR2 (46,47) have shed further light on the relationship of LTP and LTD to behavioral testing of learning and memory such as the Morris Water Maze (MWM). GluR1 knockouts have impaired LTP and LTD with normal MWM testing (48). However, on spatial working memory tasks, they are significantly impaired (48,49).

It has now been shown that AMPA-type glutamate receptors can not only participate in calcium-dependent plasticity, but can also, as a result of plasticity, alter their subunit composition (50,51). Since initial cloning studies, it has been known that GluR2-lacking receptors flux calcium (52), allowing for this to occur. Either downregulation of GluR2 or upregulation of GluR1 would potentially lead to more homomeric, calcium-permeable GluRs. This contributed to the "GluR2 hypothesis" (53,54) whereby preferential removal of GluR2 (with no changes in GluR1) can lead to AMPA-type glutamate receptors that flux calcium.

Kainate receptors have now been proposed to be involved in plasticity at mossy fiber (MFs) synapses independent of NRs (55–57). They share with NRs the cardinal feature of plasticity, namely that they can be highly permeable to the second messenger calcium (58). Kainate receptors at other synapses in the hippocampus and cortex (58–60) may also participate in the induction of plasticity in this fashion.

Glutamate Receptors and Development

Developmentally and regionally specific patterns of expression of the different glutamate receptors and their isoforms have been shown (61–63). NRs appear before GluRs, even prior to the appearance of dendritic spines (64). NR2B-containing receptors appear first with slower kinetic properties, followed by NR2A (after week 1 in the rat) with faster kinetic properties (65,66). In the rat hippocampus, GluR1 and GluR2 primarily exist in a flip isoform prior to adolescence but begin to exist in a flop isoform during adolescence (2–4 weeks of age) (63). These and other related isoforms each have unique kinetic properties (67,68). The mechanisms underlying synaptic plasticity thus vary as the animal ages (69–74) and are partly dependent on anatomic location (72,75–77). LTP remains largely dependent on NRs throughout development. However, LTD in the hippocampus develops from mostly NR-dependent forms to include NR-independent forms as the animals age (78,79). These NR-dependent and NR-independent forms are differentiated by the effectiveness of different chemical and electrical LTD inducing stimulation paradigms (78–83). Visual development coincides with changes in glutamate receptor composition at thalamo-cortical synapses (84), which has also been shown in the auditory system (85). It appears that calcium permeable or GluR2-lacking receptors are a feature only of early development (84,86–88).

Subsynaptic Machinery Regulating Insertion, Removal, and Maintenance of Glutamate Receptors

The expanding role of the subsynaptic scaffolding that interacts with glutamate receptors has been the subject of intense investigation (89–91). The central organizer appears to be PSD-95 (and related proteins), which contains a sticky tail of PDZ domains. These interactions are thought to regulate the function and targeting of glutamate receptors by tethering them at the synapse and by holding various regulatory kinases and phosphatases in proximity. NRs interact directly with PSD-95 through PDZ domains. GluRs can interact with the PDZ domains of PSD-95 (92) through TARPS (93). Interaction of GluR2 with NSF and GRIP1 seems to hold receptors in the synapse, while interaction with PICK1 removes them to extrasynaptic and subsynaptic or vesicular holding areas (94,95). GluR1 interacts (through a linkage with SAP97, a PSD-95 family member) with AKAP79/150 (96). AKAP79/150 links the complex with PKA (96,97), calcineurin, and the actin cytoskeleton (98). These interactions are thought to bring GluRs to synapses and upregulate them in LTP (99–101) and remove them in LTD (97,101–103). In LTD, the complex dissociates and moves out of dendritic spines (98). These mechanisms may be unique to the CA1 region of hippocampus where AKAP79 is primarily expressed (104).

GABAergic Ion Channels

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the adult brain. Epileptologists have been interested in this system because commonly prescribed anticonvulsant drugs, such as phenobarbital, the benzodiazepines, and to a lesser extent valproate, topiramate, and levitiracetam, reduce seizure activity by augmenting GABA receptor activity. The GABAergic system consists of three main receptor subtypes: $GABA_A$, $GABA_B$, and $GABA_C$. $GABA_A$ receptors (GABARs) are primarily located postsynaptically and mediate most of the fast synaptic inhibition in the brain. They are anion selective and gate primarily chloride, although under certain circumstances they may also gate bicarbonate. $GABA_A$ receptors are heterogeneous complexes composed of multiple protein subunits. Numerous subtypes exist for each subunit (α1–6, β1–3, γ1–3, δ, ε, π, θ, and ρ1–3). The most common in vivo GABAR subunit composition is two α, two β, and one γ subunit. There is remarkable receptor heterogeneity, with subtype combinations varying in different brain regions, cell types, and during different times in development (105–108). Different subunit subtypes and the wide variety of combinations confer

distinct functional and pharmacological properties to the GABARs (105). The γ subunit, for example, is required for $GABA_A$ receptors to be responsive to benzodiazepine-type drugs, whereas the α subunit subtype determines the type of the benzodiazepine binding site (e.g., type I or II) (109,110). Brain regions that express the highest concentration of the α1 subunit have a correspondingly high number of type I benzodiazepine binding sites and are, in turn, more sensitive to zolpidem-induced augmentation and less sensitive to zinc-induced inhibition (111–113).

$GABA_B$ receptors are G-protein-linked metabotropic receptors that are located both presynaptically and postsynaptically and are responsible for slower, more long-lasting inhibitory currents. Like $GABA_A$ receptors, they are composed of multiple subunits, primarily R1 and R2, which have additional diversity due to splice variation. Also like $GABA_A$, $GABA_B$ receptors are widely distributed in the CNS, particularly in the hippocampus, cerebellum, and thalamus. In contrast, $GABA_C$ receptors are located primarily in the retina and do not appear to play a significant role in epilepsy.

The function of the GABAergic system differs markedly in the mature and immature brain. While $GABA_A$ receptor activation results in neuronal hyperpolarization and an inhibition of cell firing in the mature brain, receptor activation results in membrane depolarization and excitation in the immature brain (114–116). The switch from GABA-mediated excitation to inhibition is related to changes in the chloride gradient that occur during the course of development (117–122). In mature neurons, the intracellular concentration of chloride is low due to the presence of KCC2-extruding transporters. When $GABA_A$ receptors are activated, chloride flows, according to its concentration gradient, into the cell; this causes membrane hyperpolarization and hence an inhibitory postsynaptic response. In contrast, intracellular concentrations of chloride are high in immature brain due to the combined effects of low KCC2 expression and the presence of NKCC1 transporters that actively carry chloride into the neuron. When $GABA_A$ receptors are activated, ion channels open, chloride flows out of the cell, and depolarization occurs. In rodents, KCC2 expression is very low during the first two postnatal weeks. By inference, it is thought that KCC2 expression is low in humans until around the end of gestation (123).

A number of laboratories have shown that depolarizing (e.g., excitatory) GABA currents are critical for the development of calcium-dependent processes, such as neuronal proliferation, migration, targeting, and synaptogenesis (124–128). In addition, there is evidence suggesting that GABAR-mediated currents also play a critical role in the generation of ictal activity in the developing brain. It has been known for some time that synchronous neuronal activity in the hippocampus can be driven by $GABA_A$ receptor activation and inhibited by $GABA_A$ receptor blockade (129). More recent evidence, however, suggests that GABAR-mediated excitation may drive ictal activity in the developing hippocampus as well (130,131).

Plasticity and Trafficking of GABAergic Receptors

During the process of epileptogenesis in animal models there are alterations in the expression and membrane localization of several GABAR subunits (α1, α4, γ2, δ) in hippocampal dentate granule neurons (132–134). These alterations, which are associated with changes in phasic and tonic GABAR-mediated inhibition, and in GABAR modulation by benzodiazepines, neurosteroids, and zinc, begin soon after SE and continue as animals become epileptic (132–135). Several laboratories have documented similar changes in GABAR subunit composition in human temporal lobe epilepsy (TLE) and in animal models of TLE (132,134,136,137). In the pilocarpine model of SE in adult rodents, GABAR α1 subunit mRNA expression decreases, α4 subunit mRNA expression increases in dentate granule cells (DGCs) of the hippocampus, and animals uniformly go on to develop the recurrent spontaneous seizures that define epilepsy (132). The change in subunit expression correlates with a decreased sensitivity to zolpidem augmentation and increased sensitivity to zinc inhibition of GABAR responses (132). Similar functional and subunit expression changes have been observed in DGCs isolated from surgically resected hippocampus from patients with intractable TLE (137). The changes in GABAR subunit expression and function in DGCs of adult epileptic animals precede the development of epilepsy and immature animals exposed to prolonged induced seizures show increased GABAR α1 subunit expression and do not subsequently develop epilepsy (138), suggesting that GABAR changes contribute to the epileptogenic process. Viral gene transfer studies demonstrating that the expression of higher α1 subunit levels inhibits development of epilepsy after SE provide further evidence in support of this (139).

Voltage-Gated Ion Channels

Generically, voltage-gated sodium channels (VGSCs) and voltage-gated calcium channels (VGCCs) are excitatory or depolarizing. VGSCs are somewhat broadly lumped as they each function similarly, with subtypes segregated to unique neuronal populations and subcompartments. However, some VGSCs have unique deactivation characteristics, often prolonged or "reverberant" resulting in unique signaling properties. VGCCs are segregated according to their biophysical properties (T, P/Q, N, and L/HVA-type) and like VGSCs are often segregated to unique neuronal populations and subcompartments. Voltage-gated potassium channels (VGKCs) are typically inhibitory or hyperpolarizing; however, depending on their voltage-dependent gating and subcellular location they can have the opposite influence on membrane potential (e.g., HCN or I_h). VGCs often share the same or similar targeting motifs and scaffolds that regulate the expression and targeting ligand-gated ion channels (140).

Neuronal Networks

Neuronal networks refer to the detailed web of connections of inhibitory and excitatory neurons within the different regions of the brain. The activation patterns and activity of different neuronal networks are thought to underlie basic brain function (141). A significant portion of experimental epilepsy research has focused on neuronal networks, specifically within the hippocampus. From a simplistic point of view, information primarily enters the hippocampus in a lamellar fashion via the dentate gyrus, travels from there to the CA3 region, then to CA1, and then out via the entorhinal cortex; however, it is

substantially more complicated than this (142,143). The CA3 has an excitatory feedback loop, which participates in normal learning, but can, however, contribute to seizure generation (144). The dentate gyrus is thought to limit CA3 excitation by acting like a filter to incoming inputs (145,146). This is due to the properties of excitatory inputs into the dentate gyrus as well as feedback inhibition within the dentate gyrus (143). Therefore, much research has focused on determining the nature of these mechanisms and how they have been potentially subverted in experimental models to result in epilepsy.

REVIEW OF TECHNIQUES

Experimental models can be divided into whole-animal (in vivo) versus in vitro studies. Whole-animal models of acquired epilepsies typically involve single or multiple treatments to the animal that produce some form of injury or stimulation that results in later development of spontaneous seizures. Examples of these induced injuries include SE (chemoconvulsant and electrical), kindling, hypoxia, and head trauma. In genetic models, a spontaneous or induced genetic mutation or deletion results in seizures that happen spontaneously. Seizure activity must be carefully defined for several reasons. First, the definition of a seizure is often extremely variable, as in the clinical literature. Second, consciousness, routinely used as a modifier in describing clinical seizures, is arbitrarily defined in most animals used. Typically, rhythmic, stereotyped, altered behavior is observed and characterized as seizure activity. As in the clinical literature, EEG has become the gold standard for correlating altered behavior with seizures, but its use is limited due to the time and labor-intensive placement of electrodes, limitations of electrode stability over time, and the fact that electrographic seizures emanating from deeper structures can be missed when recording from the cortical surface.

In Vitro Versus In Vivo Models

In vitro studies involve removal and subsequent manipulations of whole-brain structures, slices of brain structures or isolation, and culture of separated brain cells (neurons and glia). These studies allow detailed manipulations and measurements but are limited in a key way. While it is tempting to designate repetitive electrical discharges as a seizure, seizures defined in the whole animal are associated with a change in behavior or sensation which cannot be appreciated in these in vitro models and thus must be referred to as "seizure-like" events or an ictus to avoid confusion. One researcher's abnormal ictal-induced phenomena may also be interpreted as another researcher's normal activity-dependent changes. In addition, certain seizures, and their sequelae, may involve the interplay of multiple brain structures and are thus difficult if not impossible to recreate in in vitro models. Finally, key processes such as development and epileptogenesis which occur over a prolonged period of time cannot be fully studied in in vitro models as they are limited by the length of time the in vitro preparation is viable (hours to weeks).

There are dozens of in vivo and in vitro models of seizures and epilepsy and as mentioned earlier there is little consensus about which if any are the "optimal model". In reality, each model has its strengths and limitations, and the relative benefits depend on the specific question being asked. Below, we focus on the models that are in common use or emerging.

Pilocarpine and Kainate Models

The pilocarpine and lithium pilocarpine model (147) involves the systemic administration of a muscarinic acetylcholine agonist (pilocarpine) to induce a prolonged electrographic and behavioral seizure that requires cessation by benzodiazepines or barbiturates, typically after 1–2 hours, in order to prevent animal mortality. Clearly, from a clinical standpoint, this is never the cause of SE in humans. Nevertheless, it is widely used because it results in severe SE and eventually develops an epileptic phenotype with features very similar to human TLE resulting in its widespread use for studying both of these conditions.

Kainate, a glutamate analogue that is not metabolized, is either injected systemically or directly into the brain and can result in seizures lasting several hours (148,149). Clinically, kainate originates as a shellfish poison whereby human toxicity during outbreaks results in seizures and in severe cases hippocampal sclerosis (150). While this clinical situation is extremely rare, conditions involving glutamate overload that are known to be associated with seizures such as stroke, hypoxia (151,152), or infection may be mimicked to some degree by kainate administration. Similar to the pilocarpine model, because kainate results in SE, though probably not as severe as pilocarpine, and adult animals eventually develop an epileptic phenotype with features very similar to human TLE, it is widely used for studying both these conditions. In youngest animals, kainate primarily activates the hippocampus while in older animals its effects are widespread (153).

Brief Seizure Models

Pentylenetetrazole and flurothyl are GABAergic antagonists that are administered systemically or inhaled, respectively (154). They both induce relatively short seizures, with flurothyl being very brief and limited nearly to the length of exposure to the vapors. As a result, both agents are used to mimic conditions involving single or multiple brief, generalized seizures (155). The major limitations of these models are that the mechanism of seizure induction does not clearly parallel any human condition, and the animals never develop spontaneous seizures. Both agents are thought to act on all susceptible brain regions, including cortex and hippocampus (154). Electrical kindling, whereby electrodes are implanted in order to stimulate select brain regions, can also be used to study how repeated, brief seizure-like activity can influence outcomes. Depending on the stimulation protocol, eventually kindling can lead to SE. This model, however, is limited by the technicalities of long-term implantation in rodents and the fact that most kindling paradigms do not result in development of spontaneous seizures.

Clinical Models: Fever and Hypoxia/Ischemia

In models where seizures are induced in the setting of increased temperature (fever), hypoxia, and/or ischemia, the ability of these models to generalize to human pathologies is clearly evident. Hypoxia models can involve placing animals

in an environment of reduced oxygen content until seizures are observed (156,157). Other methods involve single or multiple cerebral vessel occlusions, often in combination with exposure to an environment with reduced oxygen content. Methods involving vessel occlusion are often time-intensive. These methods are then limited by the elements of hypoxia and ischemia, as these may independently influence outcomes (158). Temperature-induced seizures in developing animals (159,160) involve slowly heating the animal, typically with warmed air, until seizures are initiated. This model is gaining popularity as a model of febrile seizures but may be limited by the fact it is really a model of externally imposed hyperthermia rather than endogenous fever as occurs in the human condition.

Toxin Models

Several models involve direct infusion of toxins, compounds, or even genetic material into specific regions such as the hippocampus. These are each meant to model focal seizures or epileptogenesis, though the result can have distant effects. These include the tetanus-toxin model (161) and more recently the tetrodotoxin model (162), thought to be a model of infantile spasms or West syndrome. Knockdown of GluR2 by injection of antisense probes results in acute seizures (163). Following withdrawal of direct injection of glutamate receptor antagonists, spontaneous seizures are provoked in immature animals, while systemic injection does not cause this to happen (164).

Trauma Models

Experimental models of trauma utilizing either direct impact methods (165) or surgical undercuts (166) have been recently reviewed as models for studying the development of post-traumatic epileptogenesis and epilepsy. As head trauma is a common cause of acquired epilepsy in humans, these models seem very generalizable to human pathology. As a result, these models have been used extensively to study the efficacy of anti-epileptogenic compounds as well as the mechanisms underlying post-traumatic epileptogenesis.

In Vitro Models

In vitro methods involving brain slices or cultures use a variety of methods to induce seizure-like electrical events. These can involve perfusion of compounds that typically enhance or favor membrane excitability alone or in combination with electrical stimulation, akin to kindling. The resulting spontaneous neuronal-mediated discharges can then be recorded from groups of neurons or from individual neurons typically using electrophysiological techniques. Imaging techniques using fluorescent dyes that are able to indicate changes in membrane voltage or secondary changes due to accumulations of specific ions, such as calcium, often complement electrophysiological measurements as they are able to simultaneously record from populations of neurons that may be somewhat distant from each other. The pattern of these discharges is then interpreted either in isolation, in groups or bursts, or when the bursts cluster together as an ictus. The transitions between these types of discharges are interpreted as indicative of ictal genesis and are thought to generalize to seizure genesis. When the ictus is prolonged, this generalizes to SE. When the ability to generate an ictus becomes more facile, this is thought to generalize to epileptogenesis. Determining how excitation spreads through a slice of brain tissue is generalized to how it may spread in the intact preparation. Thus, application of anticonvulsants to an in vitro preparation has been used to determine their efficacy and precise mechanism(s) of action. In order to circumvent the issues of truly generalizable seizures, SE or epileptogenesis in vitro, brain slices are often prepared at various time points after these phenomenon have developed in vivo. Findings from hippocampal brain slices prepared from animals after experiencing an induced or spontaneous seizure in vivo allow examination of how overall synaptic transmission, plasticity, and seizure thresholds have become altered by these processes (Table 3.2).

MECHANISMS OF SE

Here, there are two basic questions: why did the seizure not stop by itself and why is SE more difficult to stop with anticonvulsants than a single seizure? Was the underlying neuronal network susceptible to this happening or did it become dynamically changed to allow its progression? Given that it has been found that the clinical situation is mimicked by the experiment in which benzodiazepines lose their potency as the seizure progresses (167), much effort has focused on the role of GABAR and inhibitory synaptic transmission (168). These questions have been approached in a variety of ways, using in vitro brain slices or in vivo models employing pilocarpine, kainate, or kindling, sometimes in combination with in vitro brain slices prepared during or after the event. Recent studies suggest that during SE, GABARs at inhibitory synapses onto granule cells of the dentate gyrus are removed from synaptic sites and moved to extrasynaptic sites and internal pools (169) in a subunit-specific manner (170). This likely minimizes their effectiveness in both self-termination of the seizure as well as the loss of effectiveness of benzodiazepines, in part mediated by loss of $\gamma 2$ subunits that modulate benzodiazepine sensitivity. These issues are complicated during development in the CA3 region of the hippocampus, where GABAergic synapses are depolarizing and thus contribute to the development of ictal activity (130,171).

The alterations in GABARs in the dentate gyrus are possibly mediated by NR activation rather than by direct activation of GABARs (170). It has been found that blocking NRs prevents the progression to drug-resistant SE (172). NRs then further contribute to the process as they are progressively recruited to synaptic sites as SE progresses (172). While in vitro studies suggest that NRs and GluRs are involved in epileptogenesis (173–175), it is possible that their contribution to this process may be mediated by their effects on SE. Reductions in GluR2 in CA1 and CA3 (176,177) 6–48 hours after SE, while implicated in cell death after SE, may have also contributed to prolonging SE, perhaps through facilitated GluR function (178). Excess glutamate, which may occur with transporter dysfunction, has been shown to lead to NR activation and seizures (179); however, this may be limited to

TABLE 3.2

ANIMAL MODEL SUMMARY

Model	Questions addressed
Pilocarpine and kainate	• SE: consequences, treatment, role of development • TLE and epileptogenesis: hippocampal networks, mechanisms and therapies • "Multihit" models
Pentylenetetrazole and flurothyl	• Multiple brief seizure models: mechanisms of epileptogenesis • Treatment of brief seizures • Role of development on long-term consequences • "Multihit" models
Temperature	• Febrile seizures in children: mechanisms and therapies • Role of development and long-term consequences • Epileptogenesis • "Multihit" models
HIE	• True "multihit" model • Role of development and long-term consequences • Epileptogenesis: mechanisms and therapies • Focal epilepsy and epileptogenesis: therapies
Toxins: tetrodotoxin and NMDA	• Infantile spasms: mechanisms & therapies • Role of development and long-term consequences • Treatment • Epileptic encephalopathies
Toxins: tetanus toxin	• Focal epilepsy and epileptogenesis: therapies • Role of development and long-term consequences
Trauma	• True "multihit" model • Role of development and long-term consequences • Focal epilepsy and epileptogenesis: mechanisms and therapies
In vitro models	• SE: consequences, treatment, role of development • Role of development • Synaptic and therapeutic mechanisms, especially when coupled with in vivo models
Genetic models	• Catastrophic epilepsies: genesis, therapy, long-term consequences • Linkage of human mutations with synaptic and electrical mechanisms in seizures and epilepsy • "Multihit" models

developing animals in which glial regulation of extracellular glutamate by transporters is immature (180). Indeed, multiple genes, including those involved in transcription, are likely regulated following SE (181).

EPILEPTOGENESIS

Epileptogenesis refers to the process by which a previously "normal" brain becomes capable of producing SRS. Animal models have typically employed prolonged SE to trigger this process; however, models of trauma and injections of toxins have also been used (see Review of Techniques). The nature and mechanisms of this process have each been richly studied. Does this happen gradually, that is, what is the significance of the latent period between trigger and first SRS? This is a critical question as it might represent a window of opportunity for intervention. What is the relationship of the sclerotic pathology, often seen in human TLE and also seen in animal models, to this process? How much of the process is due to network rewiring versus changes in neuronal and/or synaptic function? What are the signaling cascades mediating these processes and how can they be circumvented or reversed?

The appearance of SRS has been taken to indicate the end of the latent period. Enhanced excitability has been shown to gradually develop prior to the appearance of SRS (182), suggesting the end of the latent period is not a stepwise function into SRS and epilepsy. In support of this, an intensive video-EEG monitoring study has challenged the notion of the latent period by showing that the progression into SRS and epilepsy is a sigmoid function of time (183). In other words, after the first SRS, epilepsy continues to progress. Progression clearly represents a worse-case scenario that may not always be present (184). Additional work is needed to determine where

and whether there is a window for interventions to prevent this progression. Interestingly, there is a transient period following pilocarpine SE in adult animals when GABAergic inhibition becomes excitatory in some brain regions due to loss of normal chloride regulation (185), suggesting that chloride regulation may be a potential therapeutic target.

Network Reorganization

Network reorganization in the hippocampus has been extensively studied as one of the presumed origins of SRS because of similar findings in human TLE. Primarily this has focused on the output of DGC neurons and has been thoroughly reviewed (143,147,186). Excitotoxic loss of mossy cells (187) in the denate gyrus may lead to sprouting of dentate axons, known as mossy fibers (MFs). The sprouted MFs make aberrant excitatory connections locally in the dentate gyrus and distantly in CA3 creating an abnormal excitatory feedback circuit (188). These abnormal connections are further dysfunctional, with a higher probability of activation, a larger NR component (189,190), and recruitment of kainate receptors (191). These disturbances, coupled with permanent alterations in GABARs (see below), are thought to result in a circuit prone to trigger seizures in other regions, such as CA3 (143). Not without controversy, MFs and SRS have not been proven to be either necessary or sufficient for the development of TLE (7,192). Further aberrant circuits have also been described originating in CA3 (193) and CA1 (194–196). In trauma-induced epilepsy, aberrant connections are formed in the region of injury as well as the hippocampus (6,165,166). In the region of injury, discrete regions of apical dendrites have a selective overabundance of excitatory synaptic inputs and connectivity (197,198), which with alterations in membrane VGC properties (198) may also contribute to the epileptic state.

Excitotoxic cell loss (which may occur following SE or other insults) throughout the hippocampus is thought to be mediated by glutamate toxicity via GluRs (176,199) and NRs (200). Secondary reactive gliosis may also contribute to synaptic dysfunction (201,202). Loss of hilar mossy cells and other neurons mediating inhibition are thought to be critical potential contributors to the hyperexcitable steady state of the epileptic hippocampus. SE also has the paradoxical effect of inducing neurogenesis in the dentate gyrus (203). Some of the newly formed neurons may also participate in MFs or other aberrant circuitry that leads to the epileptic hippocampus (204), although the exact role of newborn neurons in epileptogenesis continues to be studied.

The role of network alterations and other causative phenomena in epileptogenesis appears to be differentially regulated depending on when in development this process is initiated. Kainate-induced SE in adult animals causes, over time, SRS, CA3 cell loss, MFs into CA3 and dentate gyrus, sprouting into CA1 stratum pyramidale and stratum radiatum, and impaired learning in memory tasks (194,205,206). Similar results are found with the pilocarpine model (147,206,207). However if animals younger than 14 days are treated with either kainate or pilocarpine, the animals do not develop spontaneous seizures (see below) (138,208,209). Single or repetitive episodes of SE in infancy caused by pilocarpine are not benign, however, and have been associated with long-term abnormalities of inhibitory neurotransmission (138). Further, single or multiple episodes of SE induced by pilocarpine at postnatal day 14 or later does result in SRS (210–212) as well as deficits in memory and learning that are inconsistently associated with cell loss and/or MFs (210,212–215). Studies in other models have not provided additional clarity regarding the association of cell loss and MFs to development of epilepsy after early-life seizures. Early-life focal administration of tetanus toxin results in a chronic epileptic state that includes memory impairment without cell loss (161) but does involve MFs (216). In contrast, repetitive flurothyl seizures in early development result in MFs, but they do not apparently result in SRS, only a reduced seizure threshold (217–219). Chronic perforant path kindling is associated with cell loss in the dentate gyrus (220). Similarly, in prolonged temperature-induced seizures, MFs gradually develops; however, reduction in seizure thresholds are seen much earlier and SRS have been reported only infrequently (221–223). Furthermore, MFs appears in a model of early-life stress, apparently unrelated to seizures (224).

Seizure or SE-Induced Alterations in Ion Channels

Early studies of in vitro brain slice models indicated that alterations in NRs with the successive prolongation of seizure-like discharges correlated with epileptogenesis (173–175). The mechanism of non-NR-mediated calcium influx via calcium-permeable GluRs is also thought to underlie cell death in adult models of seizures (176,225–227) and hypoxia (157). GluR1 upregulation has only been found in an adult model of electroconvulsive therapy (228). GluR2 "knockdown" studies have shown that downregulation of GluR2 can lead to seizures and hippocampal injury (163). Clinical evidence from pathological studies might support upregulation of GluR1 (229–231). Seizures or SE in developing animals have found either no change in GluR2 (199,232) or a downregulation of GluR2 (157,233) with no changes in GluR1 (234). Recurrent episodes of kainate-induced SE in developing animals are associated with a decrease in kainate binding (a reflection of GluRs as well as kainate receptors) in CA3 but not CA1 (209). Recurrent flurothyl seizures in developing animals have shown a long-term reduction in NRs and PSD-95 (235). Transient alteration in the properties of synaptically activated GluRs consistent with calcium permeable GluRs following hypoxic seizures in developing animals has been postulated to mediate the cascade resulting in later-life alterations in this model (236). Seizures induced by kainate in infant rats results in altered LTP, LTD, kindling and learning associated with enhanced inhibition in the dentate gyrus (237) and mechanistically linked to reduced NR2A, altered trafficking of GluR1 and increased PSD-95 (232).

In adult, epileptic animals following pilocarpine SE, GABAergic signaling is altered by specific reduction of $GABA_A$ receptor $\alpha 1$ subunits and an increase in $\alpha 4$ subunits in the dentate gyrus, resulting in a reduction in benzodiazepine sensitivity and enhanced inhibition by zinc (132). (This contrasts markedly to the developing hippocampus where pilocarpine SE does not result in epilepsy but results in an upregulation of $\alpha 1$, overall receptor numbers and enhanced benzodiazepine sensitivity [138].) Altered function of VGSCs (238,239), T-type calcium channels (240,241),

and potassium channels (242) have been described in epileptic animals and are thought to contribute to the epileptic state. In the hyperthermia model of febrile seizures, a single prolonged seizure results in permanent susceptibility to convulsants, enhanced in vitro kindling, mechanistically linked to enhancement of the voltage-gated potassium channel HCN (222,243,244).

The signaling pathways that regulate the plasticity in ion channel expression during epileptogenesis are just beginning to be elucidated. For example, recent studies have demonstrated that the mechanisms that regulate differential expression of GABAR α-subunits in hippocampus after SE include the CREB/ICER, JAK/STAT, BDNF, and Egr3 signaling pathways (245). Targeting signaling pathways that alter the expression of genes involved in epileptogenesis may provide novel therapeutic approaches for preventing or inhibiting the development of epilepsy after a precipitating insult.

SEQUELAE BEYOND EPILEPTOGENESIS

In adult models of epileptogenesis associated with cell loss and/or MFs, uniformly there is learning and memory impairment when assessed with the MWM, a behavior test used to assess spatial, long-term memory formation (246). Altered emotionality is also noted with fear conditioning (247). Mechanistically, this impairment is thought to be mediated by the anatomical damage, as similar deficits are observed in hippocampal lesion studies not associated with seizures or epileptogenesis (248). Similarly, in immature animals, abnormalities in the MWM are associated with histological changes following repetitive SE (213–215), repetitive flurothyl seizures (219,249), tetanus toxin (161), hypoxia/ischemia (250), and hyperthermia (222,243,244)-induced seizures. In models where immature animals develop SRS, there is altered emotionality (211). Furthermore, kainate insult in infancy and again later in adulthood results in more prominent memory impairment than a single insult at either time (251). In immature animals following a kainate-induced seizure, there have not been any detectable problems with the MWM or histological changes (206,252), including an absence of MFs; similar findings have been reported for repeated episodes of kainate-induced SE in immature animals (209). As adults, these animals have only subtle abnormalities in the MWM (253) and in more difficult mazes these animals have abnormalities most consistent with defective working memory (232,237,253,254); emotionality may be unaffected (253,254). Thus, permanent impairments in learning and memory are more severe in animal models when associated with significant histological abnormalities. However, significant impairments can also exist without histological abnormalities, which possibly reflect pathology limited to abnormal synaptic function isolated to the hippocampus.

GENETIC SUSCEPTIBILITY

Advances in genetics have allowed for several human epilepsy syndromes associated with single gene defects to be further characterized (255). Following determination of the analogous gene in mice, similar defects can be introduced through cloning techniques in order to better understand how epilepsy develops in these syndromes as well as determine which treatments might be more efficacious. Often, the nature of the genetic defect, whether it represents a gain or loss of function, is not clear until the altered resulting protein is expressed in an intact, cloned animal model. In the animal model of Dravet syndrome, genetic knock-in of human mutations in VGSCs (NaV1.1) results in a phenotype very similar to that seen in humans (256,257). Importantly, these studies have highlighted how the balance between excitation and inhibition is a critical modifier in this disorder (258). Similarly, genetic knock-in of human mutations in KCNQ2 and KCNQ3 has many similarities to the human phenotype of benign familial neonatal convulsions (259). Enhanced function of T-type calcium channels in thalamocortical circuits has been postulated to mediate childhood absence epilepsy. While specific mutations in T-type calcium channels have not been determined in the human condition; specific genetic targeting of enhanced expression of T-type calcium channels in this circuit have been found to mimic the human condition (260). However, genetic knock-in of human mutations in GABA receptors associated with generalized epilepsy syndromes has not resulted in phenotypes similar to the human conditions (261,262). Similarly, knock-in of human mutations in nicotinic acetylcholine receptors seen in autosomal-dominant nocturnal frontal lobe epilepsy also does not reproduce features similar to the human syndromes (263). These negative results suggest not only the complexities of genetic technologies, but also likely reflect basic underlying differences in rodent and human physiology, especially susceptibility to seizures and epilepsy.

SUMMARY

Animal models, despite their limitations, have advanced our understanding of the mechanisms of seizures and epileptogenesis. Specifically, substantial gains have been made in understanding the ability of the hippocampus and cortex to rewire themselves following insults to result in circuits capable of spontaneous seizures. Developmental models have shown how significant physiological and behavioral alterations can result without obvious histological changes. Important questions remain to be answered in further understanding the signaling pathways, genetic programs, and subsequent synaptic modifications that underlie epileptogenesis as well as the behavioral consequences of seizures. These discoveries are crucial to determine safe and effective pharmacological targets for stopping seizures and curing epilepsy and its consequences.

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