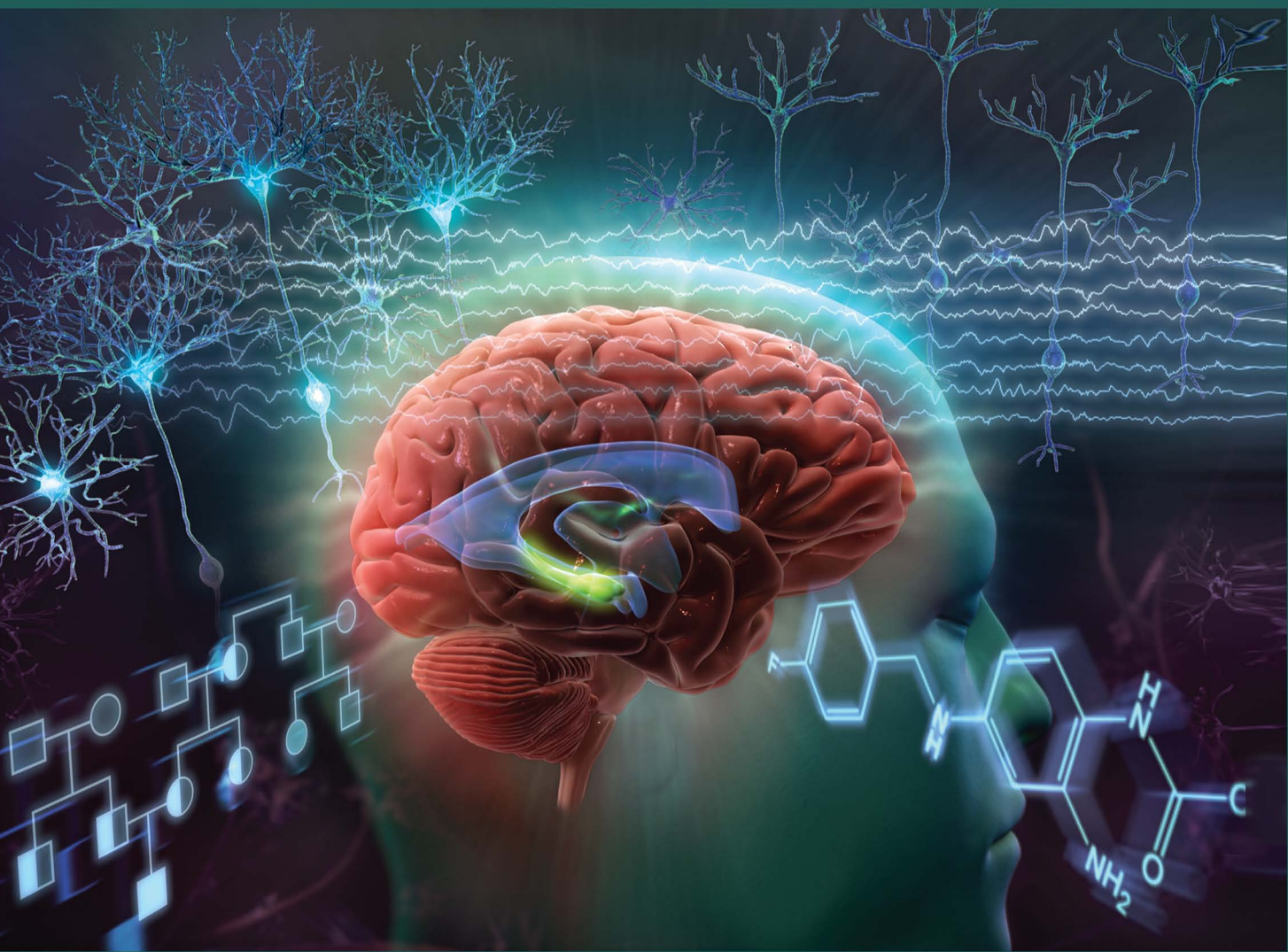
EPILEPSY

Mechanisms, Models, and Translational Perspectives



Edited by

Jong M. Rho • Raman Sankar • Carl E. Stafstrom

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Dedicated to all who have devoted their lives
to furthering our understanding of epilepsy and
advancing the care of those afflicted by this disorder

Contents

SECTION IV Alternative Therapies

SECTION V Other Modulators of the Epileptic State

SECTION VI The Future of Epilepsy Therapy

Preface

Epilepsy is an episodic neurological disorder that has afflicted humankind throughout recorded history; yet, throughout the millennia, it has never been properly acknowledged as a disease with a biological basis. In ancient times, epilepsy was referred to, somewhat ironically, as the "Sacred Disease," as it was imbued with negative references to the supernatural. Epilepsy was later believed to represent a form of demonic possession and thus resulted in social stigmatization and persecution. It was only late in the 19th century that epilepsy began its long and arduous journey to being justly recognized as a physical illness with complex pathophysiological substrates. Even today, the public is not fully apprised of the true nature of the epilepsies (as they are now considered), and efforts to expand awareness of this condition have been thwarted in large measure by deeply rooted preconceptions promulgated through the ages.

Within the last half-century, significant progress has been made in our basic understanding of the epileptic brain. Pivotal advances in drug development and surgical techniques, as well as the emergence of innovative approaches such as electrical stimulation of the nervous system, have led to a substantial reduction in the morbidity and mortality of patients with epilepsy (both children and adults). At the same time, remarkable developments in the basic neurosciences have enhanced our understanding of brain structure and function at ever finer levels of molecular, cellular, and genetic detail.

The intrinsic complexities associated with attempts at understanding normal brain structure and function lie at the heart of the challenges investigators face in deciphering the epileptic brain. The development of universally effective therapeutic approaches for epilepsy patients has been the elusive goal of clinicians and researchers since the early twentieth century. Yet, despite the availability of many new pharmacological agents within the last generation, at least one third of the people with epilepsy remain refractory to medical therapy, and an even smaller number of these individuals are potential candidates for epilepsy surgery. It is this last frustrating reality that has been the focus of many professionals in the epilepsy field.

Within the research arena, increasing focus has been placed on "translational" research (i.e., that which bridges the gap between the laboratory and patient bedside); however, effective communication and interchange between clinicians and basic researchers have been difficult to achieve on a widespread basis. It is clear that such interaction is paramount in the development of novel treatments based on a detailed knowledge of fundamental mechanisms. This volume incorporates new translational advances in bringing epilepsy therapies from the laboratory bench to the bedside and back again.

We wish to collectively thank our mentors, colleagues, students, and, most of all, our patients and their families for providing the inspiration and encouragement to help facilitate this "translational" dialog. Additionally, we thank the publisher and our families for the support they have given us throughout this project. Finally, we acknowledge the expert editorial and administrative assistance provided by Pat Roberson and Heather Milligan.

Jong M. Rho, MD
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Editors

Jong M. Rho, MD, is a senior staff scientist at the Barrow Neurological Institute and St. Joseph's Hospital & Medical Center in Phoenix, Arizona. After obtaining an undergraduate degree in molecular biophysics and biochemistry at Yale University, Dr. Rho received his medical degree from the University of Cincinnati. Following a pediatric residency at the University of Southern California Children's Hospital of Los Angeles and a neurology residency at the University of California, Los Angeles (UCLA), School of Medicine, he completed fellowships in pediatric neurology at the UCLA School of Medicine and in neuropharmacology at the National Institutes of Health (NIH). Prior to his current position, he held faculty appointments at the University of Washington and the Children's Hospital & Regional Medical Center in Seattle and the University of California at Irvine. Dr. Rho's main research interests are the mechanisms underlying the anticonvulsant and neuroprotective effects of the ketogenic diet, neuropharmacology of anticonvulsant compounds, and the study of surgically resected human epileptic tissue. His research activities have been sponsored by several NIH research grants, as well as a variety of intramural and extramural public and private sector sources. Dr. Rho has served on the editorial boards of *Epilepsia* and *Epilepsy Currents* and has been a regular reviewer for research grants submitted to the NIH. In addition to an extensive list of publications in basic science and pediatric neurology peer-reviewed journals, Dr. Rho has written numerous book chapters and edited several books and is a popular national and international guest lecturer.

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Carl E. Stafstrom, MD, PhD, is a professor of neurology and pediatrics and chief of the division of pediatric neurology at the University of Wisconsin Medical School, Madison. He received his AB from the University of Pennsylvania, followed by his MD and PhD degrees (the latter in physiology and biophysics) from the University of Washington, Seattle. After completing a residency in pediatrics at the University of Washington Children's Hospital, Dr. Stafstrom trained in adult and pediatric neurology at Tufts–New England Medical Center in Boston, followed by fellowships in neurology research, epilepsy, and clinical neurophysiology at Children's Hospital, Harvard Medical

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Introduction

Epilepsy is a common episodic neurological condition that is heterogeneous in its clinical presentation, yet characterized at a more fundamental level by the common denominators of neuronal hyperexcitability and hypersynchrony. Our understanding of epilepsy has advanced significantly over the past several decades, and the treatment options (both medical and surgical) have expanded greatly as well. Progress in the basic neurosciences has translated to ever-growing observations of molecular and cellular changes that are associated with the epileptic condition, some of which may be critical to the processes of epileptogenesis, such as the pathological changes that ensue over a latent period, ultimately resulting in spontaneous recurrent seizures and their negative consequences. Further, within the past decade, advances in molecular genetics have defined not only more clearly the role of seizure susceptibility genes and multigene influences, but also genetic mutations that are specifically linked to certain, albeit rare, epilepsy syndromes.

Nevertheless, despite such exciting developments, the clinical practice of epilepsy remains largely empiric, and few insights from the research bench have had meaningful clinical impact. The relative dearth of true "translational" (i.e., clinic to bench and back to clinic) research has hampered our ability to move beyond the limited trial-and-error approach of antiepileptic drug (AED) therapy to one that is based on a detailed understanding of how specific molecular changes might dictate truly rational and targeted pharmacotherapy. Yet, despite this, and even as the mainstay of epilepsy therapy continues to be represented by AEDs, clinicians have brought forth other novel drug approaches and nonpharmacological considerations to the treatment armamentarium, including innovative surgical interventions (e.g., deep brain stimulation).

Many books deal with the subject of epilepsy, with some focusing on clinical diagnosis and treatments and others exploring the pathological substrates of the various epilepsies. Also, some noteworthy volumes provide comprehensive overviews and discussions about both basic science and clinical topics in the field of epilepsy. However, there remains a need for additional references that integrate the most relevant research developments with clinical issues that impact directly on therapeutics. Such books would define the scientific basis of clinical practice and pose a set of challenging questions and considerations that could help shape not only the future of clinical research but also provide novel insights and avenues into more fundamental investigations that would yet again make us go "back to the bench and return to the clinic." Thoughtful clinicians, who can appreciate insights drawn from the fundamental neurosciences, can and should take a more rational approach toward the treatment of patients with epilepsy. Incorporating exciting research developments into their knowledge base will empower clinicians to "think outside the box" and to test clinical hypotheses derived from implications of basic research findings.

This volume is divided into six sections. The first section begins with a broad overview of the basic anatomic and functional substrates of seizure genesis. This is followed by half a dozen chapters highlighting novel pathogenic concepts that have both emerged and have been validated experimentally. These include (1) the role of the blood–brain barrier, (2) central nervous system inflammation, (3) the critical role of metabolism in seizure genesis, (4) the mechanistic basis of drug resistance in epilepsy, (5) complex genetics underlying epileptic conditions, and (6) the unique pathophysiological basis of certain developmental epilepsies.

Chapters in the second section are related to antiepileptic drug therapy and include a current discussion on the molecular targets of AED action and the possibility that certain AEDs may exert protective effects on the disease process itself (rather than simply suppress recurrent seizures). Other considerations in the use of AEDs, both clinically available and investigational, include an appreciation for nonsynaptic mechanisms yielding potent anticonvulsant effects, pharmacokinetic

and pharmacodynamic effects, and the genetic underpinnings of AED treatment and development. Finally, with a better understanding of drug interactions and attendant toxicities, beyond what can be established as mechanisms explaining clinical efficacy, the clinician can undoubtedly optimize the long-term care of patients suffering from epilepsy.

The third section focuses on surgical treatments for epilepsy (resective or otherwise), beginning with advances in the fields of structural and functional neuroimaging which have helped enormously in the selection of epilepsy surgery candidates and improving postsurgical outcomes. At the same time, there are emerging approaches for nonsurgical ablation of epileptic tissue and a greater understanding of the molecular and cellular bases of seizure genesis based on studies of such tissues. The fourth section reviews the variety of nontraditional therapeutic options, many of which have established efficacy in the treatment of medically refractory epilepsies (such as the ketogenic diet and the vagus nerve stimulator), but the particular clinical niches of others remain to be defined (e.g., immunomodulators, neurosteroids, herbs, botanicals).

The fifth section deals with neuroendocrine, hormonal, and biobehavioral factors that influence seizure susceptibility—information that should be incorporated into the design of treatment algorithms on an individualized basis. Finally, the last section of this book provides a glimpse of what future epilepsy therapies might look like, from novel mechanisms of drug delivery to gene and stem-cell therapies for epilepsy to seizure detection methods, which provide the pretext for highly targeted and early preventative intervention. Along these lines, the final chapter provides an overview of what has become the holy grail of epilepsy therapeutics over the past decade: the goal of preventing epilepsy itself by first identifying populations at risk and, perhaps more importantly, the critical mediators and influences that in a causal manner produce an enduring epileptic condition. Such knowledge would then be employed to intervene during critical windows of disease ontogeny, as well as during brain development.

The idea for this book was inspired by our collective desire to promote bridging of the so-called "translational divide"—that is, covering innovative treatment strategies based on scientific principles that have yet to be tested rigorously in the clinical setting but yet may provide practitioners with new approaches toward epilepsy therapeutics. It is in this spirit that we earnestly hope that the reader will benefit from this volume.

Section I

Scientific Foundations

1 Pathophysiological Mechanisms of Seizures and Epilepsy: A Primer

Carl E. Stafstrom

CONTENTS

INTRODUCTION

This chapter reviews the cellular and synaptic basis for focal and generalized seizure generation with an emphasis on ion channels and synaptic physiology. This background is useful for understanding the scientific basis of epilepsy and its treatment, as discussed in greater detail in subsequent chapters of this book.

A *seizure*, or *epileptic seizure*, is a temporary disruption of brain function due to the excessive, abnormal discharge of cortical neurons. The clinical manifestations of a seizure depend on the specific region and extent of brain involvement and may include an alteration in alertness, motor function, sensory perception, or autonomic function, or all of these. Any person can experience a seizure in the appropriate clinical setting (e.g., meningitis, hypoglycemia), attesting to the innate capacity of even a normal brain to support epileptic discharges, at least temporarily. *Epilepsy* is the condition of recurrent (two or more), unprovoked seizures, usually due to a genetic predisposition or chronic acquired pathologic state (e.g., cerebral dysgenesis, brain trauma). *Epilepsy syndrome*

refers to a constellation of clinical characteristics that consistently occur together, with seizures as a primary manifestation. Features of an epilepsy syndrome might include similar age of onset, electroencephalogram (EEG) findings, etiology, inheritance pattern, natural history of the symptoms, and response to particular antiepileptic drugs. Mechanisms leading to the generation of a seizure (*ictogenesis*) may differ from those predisposing to epilepsy, the condition of recurrent, unprovoked seizures (i.e., *epileptogenesis*) (Dichter, 2009).

A seizure is characterized by aberrant electrical activity within the brain. Such electrical activity is the net product of biochemical processes at the cellular and subcellular levels occurring in the context of large neuronal networks. Seizures often involve interplay between cortical and subcortical structures (Blumenfeld, 2003). The surface EEG is the primary clinical tool with which normal and abnormal electrical activity in the brain is measured.

At the cellular level, the two hallmark features of epileptic activity are neuronal hyperexcitability and neuronal hypersynchrony. *Hyperexcitability* is the abnormal responsiveness (e.g., lower threshold) of a neuron to excitatory input; a hyperexcitable neuron tends to fire bursts of multiple action potentials instead of just one or two. *Hypersynchrony* refers to the recruitment of large numbers of neighboring neurons into an abnormal firing mode. Ultimately, a seizure is a network phenomenon that requires participation of many neurons firing synchronously. Conventional EEG techniques can detect cortical areas exhibiting hypersynchronous discharges in the form of interictal sharp waves or spikes. Using specialized EEG recording techniques in humans and animals with epilepsy, bursts of very localized discharges have been detected that are not detected by usual EEG methods (Engel et al., 2009). These so-called "fast ripples" (250 to 600 Hz) reflect abnormal interictal discharges in restricted cortical areas which could synchronize and lead to a seizure (see Chapter 21, this volume).

CLASSIFICATION OF SEIZURE TYPES AND EPILEPSY SYNDROMES

Epileptic seizures are broadly divided into two groups, depending on their site of origin and pattern of spread (Figure 1.1). Partial seizures arise from a localized region of the brain, and the associated clinical manifestations relate to the function ordinarily subserved by that area. Focal discharges can spread locally through synaptic and nonsynaptic mechanisms or propagate distally to subcortical structures or through commissural pathways to involve the entire cortex. A seizure arising from the left motor cortex, for example, may cause jerking movements of the right upper extremity. If epileptic discharges subsequently spread to adjacent areas and eventually encompass the entire brain, a secondarily generalized seizure may ensue.

In contrast, generalized seizures begin with abnormal electrical discharges in both hemispheres simultaneously. Thus, the EEG signature of a primary generalized seizure is bilateral synchronous spike–wave discharges seen across all scalp electrodes. Primary generalized seizures critically involve reciprocal thalamocortical connections. The manifestations of such generalized epileptic activity can range from brief impairment of consciousness (as in an absence seizure) to rhythmic jerking movements of all extremities accompanied by loss of posture and consciousness (as in a generalized tonic–clonic seizure).

Although different mechanisms underlie partial vs. generalized seizures, it is useful to view any seizure activity as a perturbation in the normal balance between neuronal inhibition and neuronal excitation. Such an excitation/inhibition imbalance may occur in a localized region of brain, in multiple brain areas (which might be linked into a multinodal network), or simultaneously throughout the whole brain (McCormick and Contreras, 2001; Faingold, 2004). This imbalance is likely the consequence of a combination of increased excitation and decreased inhibition. It is useful to conceptualize excitation/inhibition imbalance as critical for seizures and epilepsy, but this notion may be overly simplistic when brain microcircuitry is analyzed in detail. In some circumstances, for example, increased inhibition can lead to enhanced hyperexcitability (see Inhibitory Synaptic Transmission section, later in this chapter) (Mann and Mody, 2008; Yu et al., 2006).

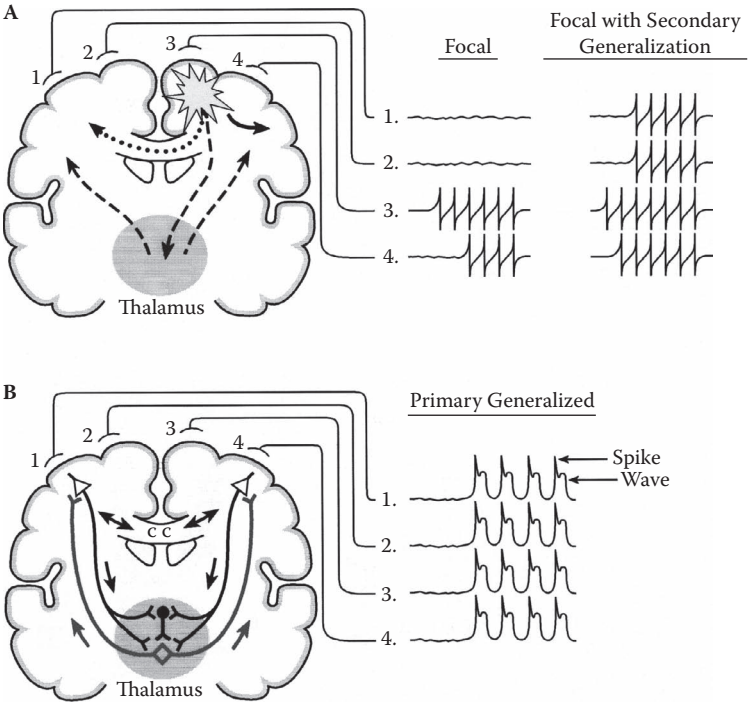

FIGURE 1.1 Coronal brain sections depicting seizure types and potential routes of seizure spread. (A) Focal area of hyperexcitability (star under electrode 3) and spread to adjacent neocortex (solid arrow under electrode 4), via corpus callosum (dotted arrow) or other commissural pathways to the contralateral cerebral hemisphere or via subcortical pathways (e.g., thalamus, upward dashed arrows). Accompanying EEG patterns show brain electrical activity under electrodes 1 to 4. Focal epileptiform activity is maximal at electrode 3 and is also seen at electrode 4 (left traces). If a seizure secondarily generalizes, activity may be seen synchronously at all electrodes, after a delay (right traces). (B) Primary generalized seizure begins simultaneously in both hemispheres. The characteristic bilateral synchronous spike–wave pattern on EEG is generated by reciprocal interactions between the cortex and thalamus, with rapid spread via corpus callosum (CC) contributing to the rapid bilateral synchrony. One type of thalamic neuron (dark neuron) is a GABAergic inhibitory cell that displays intrinsic pacemaker activity. Cortical neurons (open triangles) send impulses to both thalamic relay neurons (open diamond) and to inhibitory neurons, setting up oscillations of excitatory and inhibitory activity and giving rise to the rhythmic spike waves on EEG. (From Stafstrom, C.E., in *Epilepsy and the Ketogenic Diet*, Stafstrom, C.E. and Rho, J.M., Eds., Humana Press, Totowa, NJ, 2004, p. 3. With permission.)

It is important to recognize that epilepsy is not a singular disease but rather a heterogeneous spectrum in terms of clinical expression, underlying etiologies, and pathophysiology. As such, specific mechanisms and pathways underlying specific seizure phenotypes may vary when perturbations at a given hierarchical level lead to structural and functional changes at either higher (e.g., network) or lower (e.g., molecular) levels of analysis.

CELLULAR ELECTROPHYSIOLOGY

Regional Differences in Excitability

Brain regions differ in their intrinsic propensity to generate and propagate seizure activity, based on factors such as cell density, intrinsic membrane properties, laminar arrangement of neurons, and pattern of cellular interconnectivity. Even within the same brain region, physiological differences among various neuron types endow the region with variable excitability (Steriade, 2004).

The neocortex and hippocampus are especially prone to generating seizures. The hippocampal formation has been investigated extensively with regard to basic and epilepsy-related electrophysiological studies, and hippocampal pyramidal cells are among the most intensively studied cell types in the central nervous system. The orderly and relatively simple organization of hippocampal circuits makes them amenable for studying synaptic and nonsynaptic mechanisms relevant to seizure genesis. Furthermore, the intrinsic ability of neurons in the CA3 to fire action potentials in bursts augments the hyperexcitability of this circuit (Traub et al., 1991). Details regarding the electrophysiology of the hippocampal formation are found in classic reviews (Schwartzkroin and Mueller, 1987).

The hippocampal formation consists of the dentate gyrus, the hippocampus proper (Ammon's horn, with subregions CA1, CA2, and CA3), the subiculum, and the entorhinal cortex (Figure 1.2). These four regions are linked by excitatory, unidirectional feedforward connections. There are also some reverse projections from the entorhinal cortex to Ammon's horn and from CA3 to the dentate gyrus. The predominant forward-projecting trisynaptic circuit begins with neurons in layer II of the entorhinal cortex which project axons to the dentate gyrus along the perforant pathway, where they synapse on granule cell (and interneuron) dendrites. Granule cells, the principal cell type of the dentate gyrus, send their axons, called *mossy fibers*, to synapse on cells in the hilus and in the CA3 field of Ammon's horn. Several classes of inhibitory interneurons within the dentate hilus modulate ongoing excitatory neural activity (Lawrence and McBain, 2003). CA3 pyramidal cells project to other CA3 pyramidal cells via local collaterals, to the CA1 field of Ammon's horn via Schaffer collaterals, and to the contralateral hippocampus. CA1 pyramidal cells send their axons into the subiculum, and neurons of the subiculum project to the entorhinal cortex (as well as to other cortical and subcortical targets), thus completing the circuit. For this reason, limbic system structures such as hippocampus, subiculum, and entorhinal cortex are endowed with structural and functional features that predispose them to seizures and epilepsy (Jutila et al., 2002; Sloviter, 2008; Stafstrom, 2005).

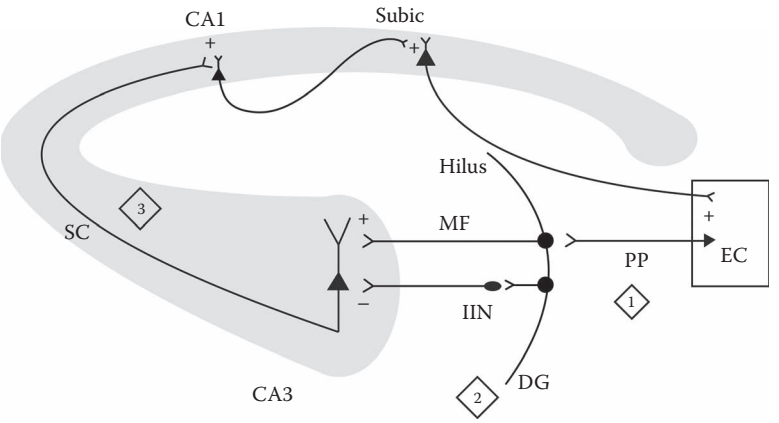

FIGURE 1.2 Schematic of hippocampal circuitry. Major pathways of excitatory transmission in the hippocampal trisynaptic pathway begin in neurons of entorhinal cortex (EC). EC neurons send axons to the dentate gyrus (DG) via the perforant path (PP) (1), where they synapse on granule cell dendrites. Dentate granule cells project their axons (mossy fibers [MF]) (2) to synapse on cells of the hilus (particularly inhibitory interneurons [IIN]) and in the CA3 field of Ammon's horn. CA3 pyramidal neurons then project to neurons of the CA1 field of Ammon's horn via Schaffer collaterals (SC) (3). Finally, CA1 neurons send projections outward through the fornix to other brain regions, including the subiculum (Subic), which then completes the circuit by exciting EC neurons. For simplicity, only feedforward excitatory projections of the classic trisynaptic pathway are depicted. Omitted are backward projections and local circuit interactions. *Note:* +, excitatory projection; –, inhibitory projection.

Ion Channels

Neuronal excitation depends on the number, type, and distribution of ion channels within the neuronal membrane. Two major types of ion channels are responsible for the inhibitory and excitatory activity comprising normal neuronal function: voltage-gated channels and ligand-gated channels. Voltage-gated sodium and calcium channels depolarize the cell membrane toward the action potential threshold. Voltage-gated potassium channels largely dampen neuronal excitation by repolarizing the membrane potential after an action potential or by opposing depolarizing conductances to keep the membrane potential below threshold. A variety of ion conductances operative in the subthreshold voltage range also sculpt neuronal activity. Voltage-gated channels are activated by membrane potential changes, which subsequently alter the conformational state of the channel and allow selective passage of charged ions through a pore.

Ligand-gated receptors include those mediating excitation (glutamate receptors) and inhibition (γ-aminobutyric acid, or GABA, receptors). A neurotransmitter (prepackaged in vesicles) is released from a presynaptic terminal (following presynaptic depolarization and calcium influx) into the synaptic cleft; the neurotransmitter then binds with selective affinity to a membrane-bound receptor on the postsynaptic membrane. Binding of a neurotransmitter to its receptor activates a cascade of events, including a conformational shift to reveal an ion-permeant pore. Passage of ions across these channels results in either depolarization (e.g., inward flux of cations) or hyperpolarization (e.g., inward flux of anions or outward flux of cations). Excitability can also be modified posttranslationally, for example, by receptor phosphorylation or by second-messenger pathways and modified gene expression.

Voltage-Dependent Membrane Conductances

Depolarizing (Excitatory) Conductances

A rapidly inactivating inward sodium conductance underlies the depolarizing phase of the action potential, and a non-inactivating, persistent sodium current can augment cell depolarization (e.g., produced by excitatory synaptic input) in the voltage range immediately subthreshold for spike initiation (Crill, 1996; Hille, 2001). Dysfunction of either the rapidly inactivating sodium current or persistent sodium current can alter neuronal excitability and enhance the propensity for epileptic firing (George, 2005; Stafstrom, 2007b).

Sodium channels consist of a complex of three polypeptide subunits; a major α-subunit forms the channel pore, and two smaller β-subunits influence the assembly and kinetic properties of the α-subunit. The shape of the action potentials is determined by the types of α- and β-subunits present in an individual neuron (Catterall et al., 2005). Many anticonvulsants act in part through interactions with voltage-dependent sodium channels, including phenytoin, carbamazepine, oxcarbazepine, felbamate, and lamotrigine (Rogawski and Löscher, 2004).

Neurons also display voltage-gated inward calcium conductances. In the hippocampus, prominent calcium currents occur in CA3 pyramidal cells, especially in dendrites, and underlie burst discharges in these cells (Wong and Prince, 1978). Activation of voltage-dependent calcium channels contributes to the depolarizing phase of the action potential and can affect neurotransmitter release, gene expression, and neuronal firing patterns. There are several distinct subtypes of calcium channels, distinguished on the basis of electrophysiological properties, pharmacological profile, molecular structure, and cellular localization (Catterall et al., 2003). The molecular structure of voltage-gated calcium channels is similar to that of sodium channels. Voltage-dependent calcium channels are hetero–oligomeric complexes comprised of a principal pore-forming α_1-subunit and one or more smaller subunits (α_2, β, γ, and δ) that are not obligatory for normal activity but can modulate the kinetic properties of the channel.

Hyperpolarizing (Inhibitory) Conductances

Depolarizing sodium and calcium currents are counterbalanced by an array of voltage-dependent hyperpolarizing currents, primarily via potassium channels. Potassium channels represent the largest and most diverse family of voltage-gated ionic channels and function to inhibit or decrease excitation in the nervous system (Hille, 2001). The prototypic voltage-gated potassium channel is composed of four membrane-spanning α-subunits and four regulatory β-subunits, which are assembled in an octameric complex to form an ion-selective pore (Gutman et al., 2005). In hippocampal neurons, potassium conductances include: (1) a leak conductance, which is a major determinant of the resting membrane potential; (2) an inward rectifier (involving the flux of other ions), which is activated by hyperpolarization; (3) a large set of delayed rectifiers, which are involved in the termination of action potentials and repolarization of the neuron's membrane potential; (4) an A-current, which helps determine inter-spike intervals and thus affects the rate of cell firing; (5) an M-current, which is sensitive to cholinergic muscarinic agonists and affects the resting membrane potential and rate of cell firing; and (6) a family of calcium-activated potassium conductances that are sensitive to intracellular calcium concentration and affect the cell firing rate and interburst interval.

Although facilitation of these hyperpolarizing conductances could be viewed as potentially anticonvulsant, none of the traditional anticonvulsants is thought to act directly and principally on voltage-gated potassium channels. By contrast, newer anticonvulsants appear to act in part by affecting potassium channel function (Rogawski, 2000; Wickenden, 2002); for example, topiramate induces a steady membrane hyperpolarization mediated by a potassium conductance (Herrero et al., 2002), and levetiracetam blocks sustained repetitive firing by paradoxically decreasing voltage-gated potassium currents (Madeja et al., 2003). Retigabine, an investigational compound with broad efficacy in animal seizure models, appears to enhance activation of KCNQ2 and KCNQ3 potassium channels (members of the so-called Kv7 family), thus increasing the effectiveness of the M-type potassium current, which acts as a brake on repetitive neuronal firing (Miceli et al., 2008; Rogawski and Bazil, 2008). This is a particularly intriguing finding given that mutations in genes encoding these proteins have been linked to a rare form of inherited epilepsy, benign familial neonatal convulsions (Biervert et al., 1998).

Synaptic Physiology

Inhibitory Synaptic Transmission

Synaptic inhibition in the hippocampus is mediated by two basic circuit configurations: (1) Feedback or recurrent inhibition occurs when excitatory principal neurons synapse with and excite inhibitory interneurons, which, in turn, project back to the principal neurons and inhibit them (i.e., a negative-feedback loop). (2) Feedforward inhibition occurs when axons projecting into the region synapse with and directly activate inhibitory interneurons, which then inhibit principal neurons. Both feedforward and feedback inhibitory circuits abound in the hippocampal formation and utilize GABA as the neurotransmitter.

GABA, the principal inhibitory neurotransmitter in the mammalian central nervous system, is a neutral amino acid synthesized from glutamic acid by the rate-limiting enzyme glutamic acid decarboxylase (GAD). GAD requires pyridoxine (vitamin B_6) as a cofactor; inherited deficiency of pyridoxine or diminished responsiveness to pyridoxine is a cause of intractable neonatal seizures (Gospe, 2006). GABA, released from axon terminals, binds to at least two classes of receptors—$GABA_A$ and $GABA_B$—which are found on almost all cortical neurons (Martin and Olsen, 2000). In addition, $GABA_A$ receptors are found on glia, where they may exert a role in the regulation of excitability (Sierra-Paredes and Sierra-Marcuño, 2007).

The $GABA_A$ receptor is a macromolecular receptor complex consisting of an ion pore as well as binding sites for agonists and a variety of allosteric modulators, such as benzodiazepines and barbiturates, each differentially affecting the kinetic properties of the receptor (Olsen and Sieghart,

2008). The $GABA_A$ receptor is a heteropentameric complex composed of combinations of several polypeptide subunits arranged in topographical fashion to form an ion channel. This channel is selectively permeable to chloride (and bicarbonate) ions. To date, seven different subunits (α, β, γ, δ, ε, π, ρ) have been described, each with one or more subtypes. Although several thousand receptor isoforms are possible from differential expression and assembly of these various subtypes, there is likely to be only a limited number of functional combinations, but the precise subunit composition of native $GABA_A$ receptors has yet to be identified. Most functional $GABA_A$ receptors follow the general motif of containing either α and β or α, β, and γ subunits with uncertain stoichiometry. Because individual subunits might be differentially sensitive to pharmacological agents, GABA receptor subunits represent potentially useful molecular targets for new anticonvulsants.

Activation of $GABA_A$ receptors on the somata of mature cortical neurons generally results in the influx of Cl^- and consequent membrane hyperpolarization, thus inhibiting cell discharge. In hippocampal cell dendrites and in the immature brain, however, $GABA_A$ receptor activation causes *depolarization* of the postsynaptic membrane. This reversal of the conventional $GABA_A$ effect is thought to reflect a reversed Cl^- electrochemical gradient, a consequence of the immature expression of the K^+–Cl^- cotransporter KCC2, which ordinarily renders GABA hyperpolarizing (Rivera et al., 1999; Staley, 2006).

In addition to $GABA_A$ receptors, "metabotropic" $GABA_B$ receptors are located on both postsynaptic membrane and on presynaptic terminals. Metabotropic receptors do not form an ion channel pore; instead, $GABA_B$ receptors act through GTP-binding proteins to control calcium or potassium conductances. Whereas $GABA_A$ receptors generate fast high-conductance inhibitory postsynaptic potentials (IPSPs) close to the cell body, $GABA_B$ receptors on the postsynaptic membrane mediate slow, long-lasting, low-conductance IPSPs, primarily in hippocampal pyramidal cell dendrites. Perhaps of greater functional significance, activation of $GABA_B$ receptors on axon terminals blocks synaptic release of neurotransmitter. It is thought that $GABA_B$ receptors are associated with terminals that release GABA onto postsynaptic $GABA_A$ receptors. In such cases, activation of $GABA_B$ receptors reduces the amount of GABA released, resulting in disinhibition (Simeone et al., 2003).

Excitatory Synaptic Transmission

Glutamate, an excitatory amino acid, is the principal excitatory neurotransmitter of the mammalian central nervous system. Glutamatergic pathways are widespread throughout the brain, and excitatory amino acid activity is critical to normal brain development and activity-dependent synaptic plasticity (Simeone et al., 2004). There are two classes of glutamate receptors: ionotropic and metabotropic. Ionotropic glutamate receptors are broadly divided into *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors, based on biophysical properties and pharmacological profiles (Dingledine et al., 1999). Each subtype of glutamate receptor consists of a multimeric assembly of subunits that determine its distinct functional properties. Glutamate receptor channel subunits are currently classified into six subfamilies based on amino acid sequence homology.

The NMDA receptor contains a binding site for glutamate (or NMDA) and a recognition site for a variety of modulators (e.g., glycine, polyamines, MK-801, zinc). NMDA receptors also display voltage-dependent block by magnesium ions (Collingridge et al., 1988). When the membrane is depolarized and the magnesium block of the NMDA receptor is alleviated, activation of the NMDA receptor results in an influx of calcium and sodium ions. Calcium entry is central to the initiation of a number of second-messenger pathways—for example, stimulation of a variety of kinases that subsequently activate signal transduction cascades leading to changes in transcriptional regulation. Activation of the NMDA receptor leads to generation of relatively slow and long-lasting excitatory postsynaptic potentials (EPSPs). These synaptic events contribute to epileptiform burst discharges, and NMDA receptor blockade results in the attenuation of bursting activity in many models of epileptiform activity (Gean, 1990; Kalia et al., 2008).

Non-NMDA ionotropic receptors are divided into α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) and kainate receptors (Dingledine et al., 1999). The AMPA receptor is responsible for the major part of the EPSP—fast-rising and brief in duration—generated by the release of glutamate onto postsynaptic neurons. In addition, the depolarization generated via AMPA receptors is necessary for effective activation of NMDA receptors. Consequently, AMPA receptor antagonists block most excitatory synaptic activity in pyramidal neurons.

Metabotropic glutamate receptors represent a large, heterogeneous family of G-protein-coupled receptors, which subsequently activate various transduction pathways, such as phosphoinositide hydrolysis and activation of adenylate cyclase and phospholipases C and D (Conn, 2003). These metabotropic receptors are important modulators of voltage-dependent potassium and calcium channels, nonselective cation currents, and ligand-gated receptors (i.e., GABA and glutamate receptors) and can regulate glutamate release. Hence, it is not surprising that they have been invoked in a wide variety of neurological processes (e.g., long-term potentiation, or LTP) and disease states (including epilepsy) (Ure et al., 2006; Wong et al., 2004). Different metabotropic glutamate receptor subtypes are specific for different intracellular processes. Although ubiquitous within the central nervous system, subtypes of metabotropic receptors appear to be differentially localized.

PATHOPHYSIOLOGY OF EPILEPTIC FIRING

Abnormal Neuronal Firing

At the neuronal cellular and network levels, there has been a concerted effort, extending over several decades, to understand the mechanisms governing the transition from normal firing to interictal epileptiform bursts to an ictal state, as well as the evolution of electrophysiological changes that terminate a seizure and underlie postictal changes (Lado and Moshe, 2008; Stafstrom, 2004). Likewise, mechanisms underlying epileptogenesis, the transition from normal brain to epileptic brain, represents both a critical knowledge gap and an opportunity for selective therapeutics (Clark and Wilson, 1999; Dichter, 2009; Dudek and Sutula, 2007). Understanding the scientific basis of both seizure generation and epileptogenesis requires correlated laboratory and clinical investigations. Much of our understanding of epilepsy mechanisms comes from *in vivo* animal models and *in vitro* electrophysiological studies.

As discussed earlier, consideration of the imbalance between excitatory and inhibitory factors helps to guide the approach to the mechanisms involved. Mutations have been identified in genes coding for ion channel proteins in both humans and animal models, many of which express seizures as a phenotype. These so-called epilepsy "channelopathies" represent a window for dissecting mechanisms of seizure genesis (Helbig et al., 2008; Reid et al., 2009). In addition, mutations in genes coding for proteins responsible for neurotransmitter transport and biogenesis, as well as receptor trafficking to the correct membrane location, are expanding the repertoire of epilepsy mechanisms (Hirose, 2006; Macdonald and Kang, 2009).

Figure 1.3 depicts EEG and intracellular changes that can be seen in normal, interictal, and ictal states. In the normal situation, action potentials (which represent "all-or-none" events) are generated when the neuronal membrane potential reaches the threshold for firing (approximately –40 mV). These discharges may influence the activity of adjacent neurons through electrical field (i.e., ephaptic) or synaptic mechanisms, resulting in an EPSP. Nearby inhibitory interneurons may also be activated, after a brief delay, giving rise to an IPSP. The activity recorded in the target neuron (neuron 2 of Figure 1.3B) will reflect the temporal and spatial summation of both EPSP and IPSP inputs. When extrapolated to multiple synaptic contacts, the sculpting of individual cellular responses modulated by various degrees of inhibition can be envisioned. Further, when considering that a single inhibitory interneuron can connect with hundreds or thousands of pyramidal

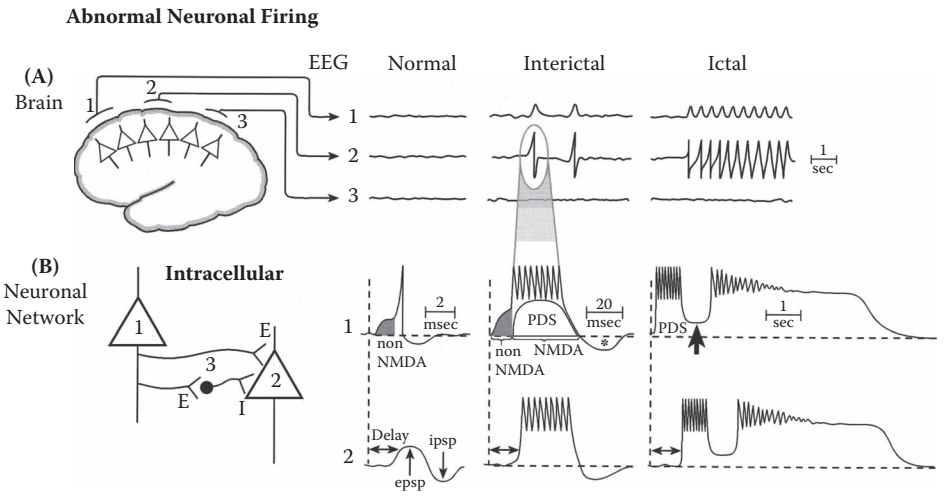

FIGURE 1.3 Abnormal neuronal firing at the levels of (A) the brain and (B) a simplified neuronal network, consisting of two excitatory neurons (1 and 2) and an inhibitory interneuron (filled black circle, 3). EEG (top set of traces) and intracellular recordings (bottom set of traces) are shown for the normal (left column), interictal (middle column), and ictal (right column) conditions. Numbered traces refer to like-numbered recording sites. Note time-scale differences in different traces. (A) Three EEG electrodes record activity from superficial neocortical neurons. In the normal case, activity is low voltage and desynchronized (neurons are not firing together in synchrony). In the interictal condition, large spikes are seen focally at electrode 2 (and to a lesser extent at electrode 1, where they might be termed "sharp waves"), representing synchronized firing of a large population of hyperexcitable neurons (expanded in time below). The ictal state is characterized by a long run of spikes. (B) At the neuronal network level, the intracellular correlate of the interictal EEG spike is the paroxysmal depolarization shift (PDS). The PDS is initiated by a non-NMDA-mediated fast EPSP (shaded area) but is maintained by a longer, larger NMDA-mediated EPSP. The post-PDS hyperpolarization (asterisk) temporarily stabilizes the neuron. If this post-PDS hyperpolarization fails (right column, thick arrow), ictal discharge can occur. The lowermost traces, recordings from neuron 2, show activity similar to that recorded in neuron 1, with some delay (double-headed horizontal arrow). Activation of inhibitory neuron 3 by the firing of neuron 1 prevents neuron 2 from generating an action potential (the IPSP counters the depolarization caused by the EPSP). If neuron 2 does reach the firing threshold, additional neurons will be recruited, leading to an entire network firing in synchrony (seizure). (From Stafstrom, C.E., in *Epilepsy and the Ketogenic Diet*, Stafstrom, C.E. and Rho, J.M., Eds., Humana Press, Totowa, NJ, 2004, p. 3. With permission.)

neurons, it is straightforward to see how hypersynchronous behavior can be influenced by even a single cell. For localized hyperexcitability to spread to adjacent areas, the epileptic firing must overcome the powerful inhibitory influences that normally keep aberrant excitability in check (i.e., the "inhibitory surround").

Paroxysmal Depolarization Shift

The intracellular correlate of the focal interictal epileptiform discharge on EEG is known as the paroxysmal depolarization shift (PDS) (Ayala, 1983). The PDS is seen when recording changes in the membrane potential of a single neuron with a microelectrode while simultaneously recording a focal spike on EEG (Figure 1.3B). Initially, there is a rapid shift in the membrane potential in a depolarizing direction initiated by synaptic forces, followed by a burst of repetitive action potentials on a depolarizing plateau potential lasting several hundred milliseconds (Johnston and Brown, 1984). The initial depolarization is mediated by non-NMDA glutamate receptors (i.e., AMPA receptors), while the sustained depolarization is a consequence of NMDA receptor activation. Afterward,

the PDS terminates with a repolarization phase, primarily as a consequence of inhibitory potassium and chloride conductances, carried by voltage-gated potassium channels and GABA receptors, respectively. Of note, there is a prolonged period of hyperpolarization following the PDS, again mediated by inhibitory conductances, constituting a refractory period. Failure of mechanisms to terminate a PDS could lead to massive prolongation of the abnormal neuronal discharge—that is, a seizure.

Synchronizing Mechanisms

The hippocampus normally displays robust neuronal synchronization. Sharp waves, dentate spikes, theta activity, 40-Hz oscillations, and 200-Hz oscillations are all forms of neuronal synchronization that can be recorded in various regions of the hippocampal formation (Buzsáki and Draguhn, 2004). Thus, synchronization of neuronal activity appears to be how the brain performs many of its normal functions; however, exaggerated neuronal synchronization is a hallmark of epilepsy. In addition, normal forms of synchronized activity that do not trigger seizures in normal tissue might trigger epileptiform discharges in a brain region that has undergone selective neuronal loss, synaptic reorganization, or changes in receptor expression.

In the hippocampus, synchronizing mechanisms include inputs from subcortical nuclei as well as intrinsic interneuron-mediated synchronization. For example, high-amplitude theta activity (4 to 7 Hz) is a salient feature of the hippocampus. The theta rhythm represents synchronized activity of hippocampal neurons and is largely dependent on input from the septum (Buzsáki and Draguhn, 2004). Subcortical nuclei such as the septum have divergent inputs that target hippocampal interneurons. In turn, the divergent axon projections of interneurons, and the powerful effect of the $GABA_A$-receptor-mediated conductances that they produce, enable interneurons to entrain the activity of large populations of principal cells. These characteristics make interneurons a very effective target for subcortical modulation of hippocampal principal cell activity. In addition, mutual inhibitory interactions among hippocampal interneurons can produce synchronized discharges (Jefferys et al., 1996).

Recurrent excitatory circuits are another basis for neuronal synchronization in the hippocampus. Recurrent excitatory collaterals are a normal feature of the CA3 region. CA3 pyramidal cells form direct, monosynaptic connections with other CA3 pyramidal cells. These recurrent excitatory interactions contribute to the synchronized burst discharges that characterize this region of Ammon's horn (Traub and Miles, 1991). In the epileptic temporal lobe, synaptic reorganization and axonal sprouting lead to aberrant recurrent excitation, providing a synchronizing mechanism in other parts of the hippocampal formation, including the CA1 region, subiculum, entorhinal cortex, and dentate gyrus. In the dentate gyrus of normal hippocampus, for example, granule cells form few, if any, monosynaptic contacts with neighboring granule cells. In epileptic hippocampus, mossy fiber sprouting results in direct excitatory interactions among granule cells (Figure 1.4) (Nadler, 2003; Sutula, 2002).

Finally, mechanisms independent of chemical synaptic transmission might synchronize hippocampal neuronal firing under some circumstances (Dudek et al., 1998); for example, gap junctions allow electrical signals to pass directly between cells (Traub et al., 2004). Gap junctions are upregulated in epileptic brain, and blockade of gap junctions significantly affects the duration of seizure activity (Nemani and Binder, 2005). Another example is illustrated by electrical field effects generated through current flow within the extracellular space. The potential synchronizing effect of such "ephaptic" interactions suggests that manipulations altering the extracellular space volume (thus affecting current flow through this compartment) can impact epileptogenicity (Schwartzkroin et al., 1998). In addition, changes in extracellular ion concentrations can affect excitability. Increases in extracellular potassium concentration have long been known to affect epileptogenic excitability and synchronization (Fröhlich et al., 2008; Traynelis and Dingledine, 1988).

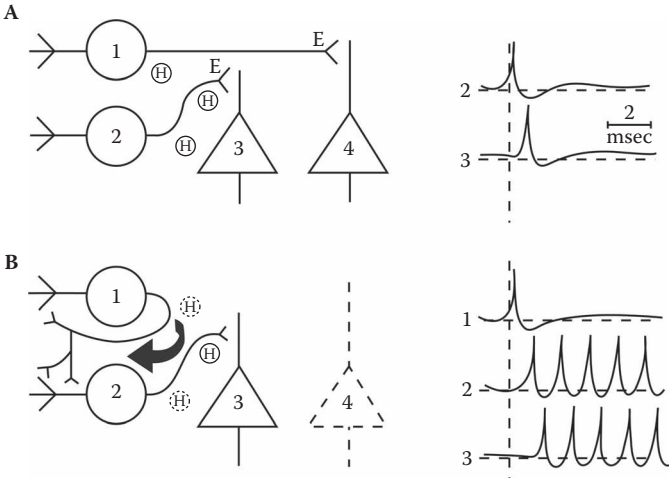

FIGURE 1.4 Simplified depiction of axonal sprouting in the hippocampal dentate gyrus. (A) Normal situation. Left: Dentate granule neurons (1, 2) make excitatory synapses (E) onto dendrites of hippocampal pyramidal neurons (3, 4). Right: Activation of dentate neuron 2 causes single action potential in pyramidal neuron 3 after a synaptic delay. (B) As a consequence of status epilepticus, many pyramidal neurons die (4, dashed outline), leaving axons of dentate neuron 1 without a postsynaptic target; those axons then "sprout" and innervate the dendrites of other granule neurons (thick curved arrow), creating the substrate for a hyperexcitable circuit. Now, when neuron 1 is activated, multiple action potentials are fired in neuron 2 and, therefore, in neuron 3 (right traces). As described in the text, this diagram is simplified and, in fact, numerous types of interneurons in the dentate hilus (labeled H) are also involved in the outcome of seizure-induced synaptic plasticity. The resultant circuit function will depend on the character (excitatory or inhibitory) and connectivity of these interneurons. (From Stafstrom, C.E., in *Epilepsy and the Ketogenic Diet*, Stafstrom, C.E. and Rho, J.M., Eds., Humana Press, Totowa, NJ, 2004, p. 3. With permission.)

Glial Mechanisms for Modulating Epileptogenicity

The critical role of glia in epilepsy is now receiving due attention (Wetherington et al., 2008). Because the ionic balance between intracellular and extracellular compartments is altered after neuronal activity (especially after sustained repetitive discharges seen with seizures), there must exist mechanisms to restore ionic homeostasis. Otherwise, even normal neuronal activity would cease.

Astrocytes are perhaps most closely associated with regulation of extracellular potassium levels (potassium buffering), as it is clear that glia membranes are preferentially permeable to potassium. A variety of inwardly rectifying K^+ channels provide an appropriate means for potassium uptake, and the association of glial endfeet with brain microvasculature provides a convenient "sink" for potassium release. Glial membrane potential changes are directly correlated with changes in extracellular potassium, and blockade of potassium channels selective to glia results in neuronal hyperexcitability. Thus, it seems certain that glia help to modulate neuronal discharges through their regulation of extracellular potassium.

Despite the evidence that glia participate in potassium regulation, glia play an even more important role in the maintenance of neuronal excitability by transporting glutamate (released from neuronal terminals to excite other neurons) out of the extracellular space. Glia are uniquely equipped for this role, having at least two powerful glutamate transport molecules in their membranes (Rothstein et al., 1994). Rapid and efficient removal of extracellular glutamate characterizes normal healthy brain tissue and is essential because residual glutamate would continue to excite surrounding neurons. Indeed, blockade of glutamate transporters (or "knockout" of the genes for these transport proteins) results in epilepsy or excitotoxicity (Tanaka et al., 1997).

Glia contribute to modulation of excitability in a number of other ways. First, they play a critical role of regulating extracellular pH via a proton exchanger and bicarbonate transporter mechanisms. Even low levels of neuronal activity create significant pH transients. Further, pH modulates receptor function, particularly that of the NMDA receptor, which appears to play such an important role in epileptic discharge. Second, glia are also now thought to release powerful neuroactive agents into the extracellular space. Studies have shown that glial glutamate release can excite neighboring neurons (Tian et al., 2005), and other glia-related factors, such as the cytokine interleukin-1 (IL-1), can have profound anticonvulsant efficacy (Vezzani et al., 2008).

Physiology of Absence Epilepsy

Absence seizures represent a subtype of generalized-onset seizures with a distinct pathophysiological substrate. An absence seizure is characterized by a temporary loss of awareness and usually a sudden cessation of motor activity. These seizures are generally short lived (lasting less than 20 seconds in most cases), begin without an aura, and end abruptly without postictal changes.

The 3-Hz generalized spike–wave discharges seen with an absence seizure reflect a widespread, phase-locked oscillation between excitation (i.e., spike) and inhibition (i.e., slow wave) in mutually connected thalamocortical networks (Huguenard and McCormick, 2007; McCormick and Contreras, 2001). As depicted in Figure 1.5, neurons of layer VI of the neocortex have excitatory projections to thalamic relay (TR) neurons as well as to inhibitory GABAergic neurons comprising the nucleus reticularis thalami (NRT). In turn, excitatory outputs of TR neurons impinge upon the apical dendrites of layer VI pyramidal neurons in neocortex. This so-called thalamocortical relay is a critical substrate for the generation of cortical rhythms and is influenced by sensory inputs (e.g., from the retina). In addition, ascending projections from several brainstem nuclei, including cholinergic, noradrenergic, and serotonergic inputs, modulate thalamocortical activity (Chang and Lowenstein, 2003). This reciprocal circuitry, which is responsible in large part for normal EEG oscillations during wake and sleep states, could become overactive to generate generalized spike–wave discharges or could be dampened to reduce or eliminate spontaneous cortical rhythms. Further, the anatomy implies that spike–wave discharges can be interrupted at either cortical or thalamic levels.

Although multiple ionic conductances are involved in rhythmic pacemaking activity, two specific channels are believed to play a key role in regulating thalamocortical activity. The first is a subtype of voltage-gated calcium channel known as the "low-threshold" or T-type calcium channel (Perez-Reyes, 2003). The channel is so named because it can be activated by small membrane depolarizations. In many neurons, calcium influx through these channels triggers low-threshold spikes which in turn activate a burst of action potentials (McCormick and Contreras, 2001). Such an excitatory burst is believed to underlie the "spike" portion of a generalized spike–wave oscillation.

The second important ion channel involved in the regulation of thalamocortical rhythmicity is the hyperpolarization-activated cation channels (HCN channels), responsible for the so-called H-current. HCN channels, densely expressed in the thalamus and hippocampus, are activated by hyperpolarization and produce a depolarizing current carried by an inward flux of Na^+ and K^+ ions (Robinson and Siegelbaum, 2003; Wahl-Schott and Biel, 2009). This depolarization helps to bring the resting membrane potential toward threshold for activation of T-type calcium channels which in turn produces a calcium spike and a burst of action potentials. HCN channels are highly expressed in dendrites and to a lesser extent in the soma.

Unlike other voltage-gated conductances that can be labeled either inhibitory or excitatory, H-currents are both inhibitory and excitatory (Poolos, 2004). HCN channels possess an inherent negative-feedback property; hyperpolarization produces an activation of HCN channels, which then leads to depolarization which then deactivates these channels. The net effect of HCN channel activation is a decrease in the input resistance of the membrane, which is the voltage change produced by a given synaptic current. The H-current tends to stabilize the neuronal membrane potential toward the resting potential against both hyperpolarizing and depolarizing inputs. HCN channels

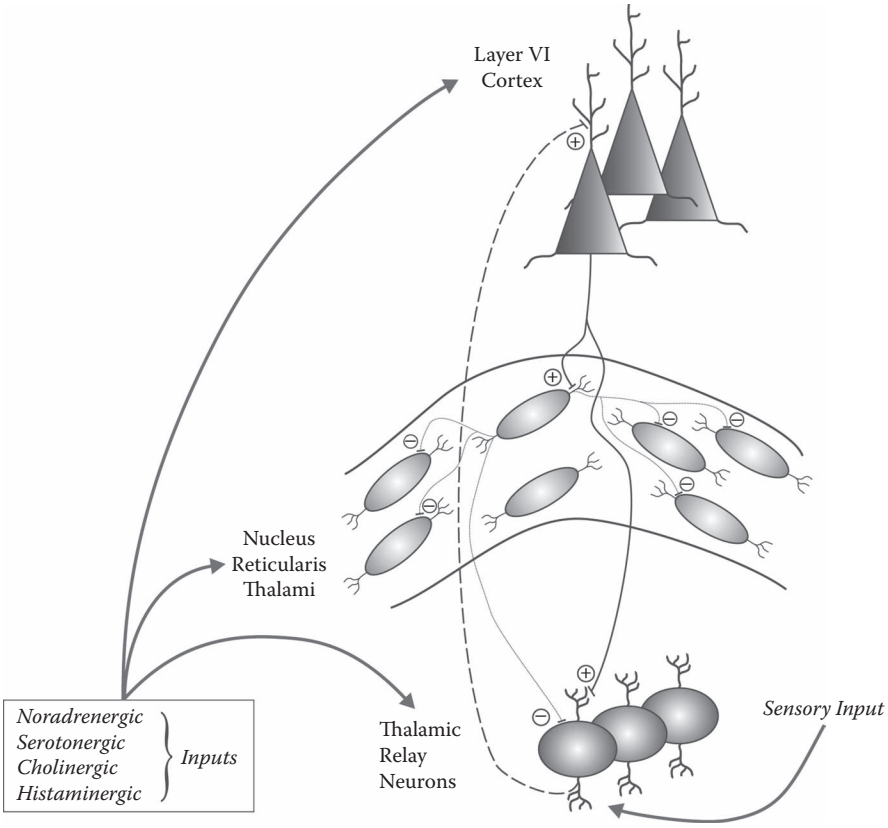

FIGURE 1.5 Thalamocortical circuitry believed to form the basis of normal oscillatory rhythms that, when perturbed, can produce generalized spike–wave discharges seen with absence seizures. This circuitry involves excitatory projections from layer VI neocortical pyramidal neurons onto both thalamic relay (TR) neurons and inhibitory neurons comprising the nucleus reticularis thalami (NRT). TR neurons, in turn, send excitatory axons back to the neocortex. Activation of NRT neurons results in recurrent inhibition to both adjacent neurons as well as TR neurons. The neurotransmitters at the excitatory (++) and inhibitory (–) synapses are thought to be glutamate and γ-aminobutyric acid (GABA), respectively. Low-threshold (T-type) calcium channels and hyperpolarization-activated cation (HCN) channels in both TR and NRT neurons help regulate intrinsic rhythmicity. Extrinsic modulatory influences to this circuitry include inputs from both forebrain and brainstem nuclei, as well as sensory inputs from other thalamic nuclei. (From Rho, J.M. and Stafstrom, C.E., in *Pediatric Neurology: Principles and Practice*, 4th ed., Swaiman, K.F. et al., Eds., Mosby, Philadelphia, PA, 2006, p. 991. With permission.)

also appear to be involved in other types of epilepsy such as febrile seizures and temporal lobe epilepsy (Chen et al., 2001; Shin et al., 2008).

Anticonvulsants known to be clinically effective against absence seizures (e.g., ethosuximide and valproic acid) can block T-type calcium currents (Coulter et al., 1989). The relevance of HCN channels in the pathogenesis of absence seizures was underscored by the demonstration that lamotrigine, an anti-absence agent, enhanced activation of dendritic H-currents in hippocampal pyramidal neurons (Poolos et al., 2002). Furthermore, antagonists of $GABA_B$ receptors and dopaminergic agonists can also interrupt abnormal thalamocortical discharges in experimental absence epilepsy models (Snead, 1995). Finally, $GABA_B$ receptors are involved in mediating long-lasting thalamic IPSPs involved in the generation of normal thalamocortical rhythms, whereas brainstem monoaminergic projections disrupt these rhythms. Thus, absence seizures appear to result from the combined dysfunction of T-type calcium channels, HCN channels, and $GABA_B$ receptors, as well as other pathophysiological mechanisms.

Increased Seizure Susceptibility of the Immature Brain

Seizure incidence is highest during the first decade and especially during the first year of life (Hauser, 1994). Multiple physiological factors contribute to the increased susceptibility of the developing brain to seizures (Ben-Ari and Holmes, 2006; Sanchez and Jensen, 2001; Silverstein and Jensen, 2007; Stafstrom, 2007a). Each factor alters the brain excitatory/inhibitory balance in favor of enhanced excitation and involves a multiplicity of substrates and mediators, including ion channels, neurotransmitters and their receptors, structural changes in the maturing brain, and ionic gradients. Seizure propensity in the very young involves a complex interplay between the timing of these cellular and molecular changes. In addition, excitability in the developing brain varies by brain region and cell type. Much of this information is derived from experimental epilepsy models in rodents, in which a window of heightened excitability in the second postnatal week gives rise to a lowered seizure threshold. This period of hyperexcitability in the rodent is approximately analogous to the first year of life in the human infant (Avishai-Eliner et al., 2002).

For many reasons, the excitatory/inhibitory balance in the brain changes dramatically over the course of early development. The disadvantage of these physiological adaptations is that the brain is especially vulnerable to hyperexcitability and seizure generation during a critical window of development. Nevertheless, these neurophysiological idiosyncrasies of early brain development also provide the opportunity for producing novel, age-specific therapies, some of which are undergoing development (Dzhala et al., 2008; Mazarati et al., 2008). These topics are explored in further depth by Weisenberg and Wong in Chapter 7 in this volume.

SUMMARY

Epileptic seizures arise from a multiplicity of factors that regulate neuronal excitability and synchrony. Although much has been learned about the molecular and cellular alterations that produce or accompany seizure activity, we have yet to fully integrate these findings with our (largely phenomenological) electroclinical observations and treatment responses in experimental animal models of epilepsy, as well as in humans. In dissecting the basic neurophysiology of epilepsy, it is tempting to invoke causality on any identifiable alteration that could theoretically enhance neuronal excitation; however, investigators frequently discover that it is not easy to identify the critical mediators and pathways in the processes of ictogenesis and epileptogenesis. Ultimately, seizures are a reflection of a complex array of perturbations occurring at multiple hierarchical levels of cell structure and function and, perhaps more importantly, as yet unpredictable products of large neuronal network activity. The subsequent chapters of this volume explore many of these topics in greater detail.

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2 Blood–Brain Barrier, Blood Flow, Neoplasms, and Epilepsy: The Role of Astrocytes

Luca Cucullo, Nicola Marchi, Vincent Fazio, Minh-Tri Nguyen, and Damir Janigro

CONTENTS

INTRODUCTION

Glial cells are non-neuronal cells that play a major supportive and modulatory role in a variety of brain functions, spanning a multiplicity of functions from physical support for neurons to regulation of the brain environment. In general, glial cells help maintain the homeostasis necessary for neuronal function. Glial cells also play an important developmental role by guiding neuronal migration during early stages of maturation and by modulating the growth of axons and dendrites. Although different glial cells perform different functions, the astrocyte family is the main regulator of neurovascular interactions, the primary focus of this review.

At the brain microvascular level, astrocytes play a primary role in the differentiation of cerebrovascular endothelial cells into blood–brain barrier endothelium. Astrocytes also contribute to the modulation of mature blood–brain barrier (BBB) function and activity. At the level of arterioles and venules, astrocytes regulate vascular tone by the release of vasoactive substances, thus contributing to cerebral blood flow regulation. In general, it is reasonable to assume that a healthy astrocyte is a reflection of a properly functioning brain. Conversely, the phenomenon of *reactive gliosis* is a sign of pathophysiological derangements that may revert or progress further into neurological disorders.

Consistent with their role as regulators of brain homeostasis, glial cells also play a major modulatory role in inflammation by releasing proinflammatory cytokines that act as chemotactic agents for peripheral immune cells. Glia also facilitate leukocyte extravasation into the perivascular space and even infiltrate into the brain parenchyma. Whereas mature glia undergo mitosis reluctantly, misguided proliferation of glia can lead to the development of devastating brain tumors such as high-grade gliomas.

In this chapter, we cover various aspects of glial cell functions, starting from their role in the development and regulation of the BBB. We also describe their involvement in the regulation and maintenance of brain homeostasis, with particular focus on drug resistance, drug metabolism, and inflammation. Finally, we discuss the pathogenesis of brain tumors originating from glial cells and some recent views on the correlations among electrical excitability, cell proliferation, and drug resistance that can be exploited for the development of novel therapeutic strategies.

ASTROCYTES AND THE BLOOD–BRAIN BARRIER

Glial cells are numerically the predominant cell type in the brain, and the glial/neuron ratio increases dramatically with brain complexity and size (Oberheim et al., 2006). Astrocytes, a specific subtype of glial cell, play an important role in regulating cerebral ionic homeostasis (Allen and Barres, 2009) and transmitter regulation (Newman, 2003). They also contribute to the maintenance of the BBB (Ballabh et al., 2004) and provide structural as well as metabolic support of neuronal cells—for example, by providing the glucose–lactate shuttle (Magistretti, 2006). At the vascular level, astrocytes extend larger processes, known as *endfeet*, whose terminations cover 99% of the abluminal vascular surface of capillaries, arterioles, and venules present in the cerebrovascular network (Simard et al., 2003). At the brain microcapillary level, these cells become one of the main building blocks of the BBB, a highly specialized dynamic and functional interface between the blood and the brain that plays a critical role in controlling and modulating the homeostasis of the central nervous system (CNS). The BBB greatly restricts the permeability of the paracellular pathways and regulates the passage of bloodborne substrates (e.g., ions, nutrients, amino acids) between the blood and the brain. The BBB also provides a defensive line against the passage of potentially harmful xenobiotic substances and modulates the immune response both at vascular and perivascular levels (see Figure 2.1).

The differentiation of vascular endothelial cells into a BBB phenotype and the induction of BBB properties are heavily dependent upon their association with perivascular glial cells (Prat et al., 2001; Utsumi et al., 2000) or astrocytes. These glia regulate protein expression, modulate endothelium differentiation, and are critical for the induction and maintenance of tight junctions and BBB properties (Grant and Janigro, 2004; Zlokovic, 2008). In fact, when BBB endothelial cells are isolated from their original environment and cultured *in vitro*, they start dedifferentiating into a phenotype similar to that of peripheral vascular endothelial cells. This occurs despite the fact that the genetic characteristics of the original phenotype are maintained (Reinhardt and Gloor, 1997). Conversely, when non-BBB endothelial cells are cocultured under exposure to intraluminal flow and in the presence of abluminal glia, they start developing BBB properties. This is clearly demonstrated by the development of higher transendothelial electrical resistance (Cucullo et al., 2002), lower permeability to paracellular markers (Stanness et al., 1997), the polarized expression of transporters such as glucose transporter type 1 (GLUT-1) (McAllister et al., 2001), amino acid transporters (Grant et al., 2003; Parkinson et al., 1998), and functional tight junctions (Stanness et al., 1997), which form a diffusion barrier that selectively excludes most bloodborne and xenobiotic substances from entering the brain. Other markers typically related to the formation of a functional BBB, including gamma-glutamyl transpeptidase (γGTP), transferrin receptor, and P-glycoprotein, are also upregulated in endothelial cells when cocultured with astrocytes (Virgintino et al., 1998).

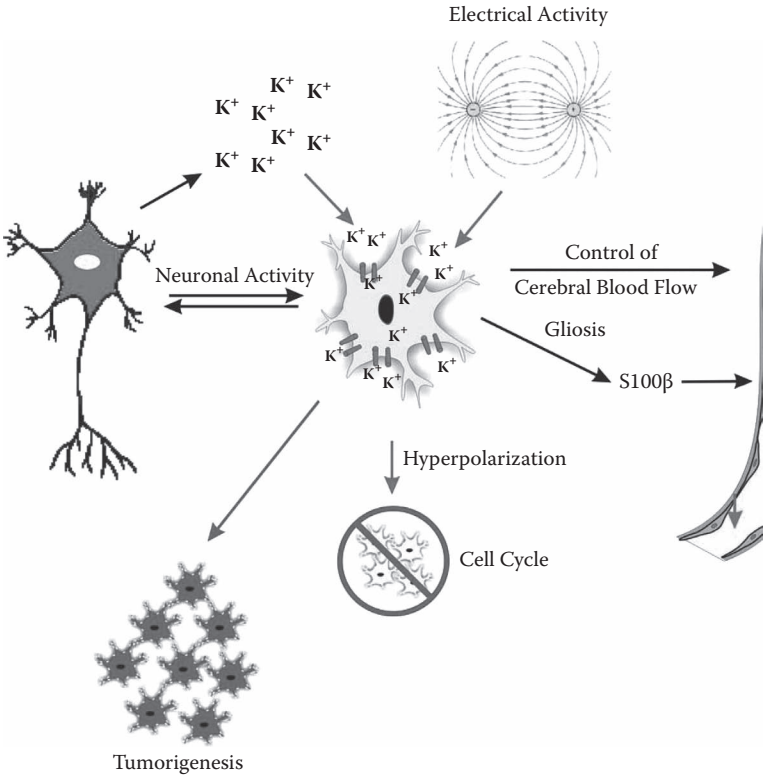

FIGURE 2.1 Astrocytes are essential players in normal and abnormal brain. On the one hand, they protect against insults, control synaptic transmission and perhaps blood flow, and also contribute to BBB maintenance. On the other hand, astrocytes also give rise, albeit reluctantly, to potentially deadly neoplasms.

Another important element of the BBB is the basal lamina, or extracellular matrix (ECM), whose functions range from mechanical support for cell adhesion and transmembrane migration of leukocytes (Zlokovic, 2008) to the regulation of cellular modulation across the BBB (Wolburg et al., 2009). The basal lamina is generated and maintained by perivascular astrocytes in cooperation with the BBB endothelium (del Zoppo and Milner, 2006; Wolberg et al., 2009).

DO ASTROCYTES REGULATE CEREBRAL BLOOD FLOW?

Functional hyperemia, which is the active process of vasodilation in response to the rising metabolic demands of increased neuronal activity, is a critically important element that is fundamental to normal brain function (Kuschinsky, 1997). Recent studies have proposed that astrocytes play a dynamic role at the neurovascular level (Volterra and Meldolesi, 2005); the anatomical correlates of this important function are the strategically located contact with the neuronal synapses on one end and a link to the vascular network by means of the astrocytic endfeet (Ransom et al., 2003; Volman et al., 2007).

The primary role of astrocytes as neurovascular elements is critical to coupling the metabolic demands of neurons (Magistretti, 2006) to cerebral blood vessel responses where variations in neuronal activity trigger localized changes in blood flow (Haydon and Carmignoto, 2006; Takano et al., 2006; Zonta et al., 2003). In this process, the release of glutamate through the stimulation of metabotropic glutamate receptors (mGluRs) raises intracellular Ca^{2+} levels in astrocytic endfeet

and leads to changes in the vascular tone of neighboring intracerebral arterioles. For example, the increase in Ca^{2+} in astrocyte endfeet promotes the opening of astrocytic K_{Ca} channels and the release of K^+, which could then act on vascular inward rectifier potassium (K_{ir}) channels to produce vasodilation; however, the vascular response observed in conjunction with an elevation of intracellular Ca^{2+} levels in astrocytic endfeet is equivocal and can lead to vasodilation or vasoconstriction (Gordon et al., 2007). A similar bell-shaped dose response is seen when potassium is directly applied to the vessel (Faraci and Heistad, 1993; Janigro et al., 1996; Nguyen et al., 2000). In the case of K_{Ca} channels, the elevation of intracellular levels of Ca^{2+} leads to the production of arachidonic acid (AA) by Ca^{2+}-sensitive phospholipase A_2 (PLA_2). The conversion of AA to 20-hydroxyeicosatetraenoic acid (20-HETE) leads to vasoconstriction, and the conversion to epoxyeicosatrienoic acid (EET) or prostaglandin E_2 (PGE_2) leads to vasorelaxation. Other factors that play an important role in determining the propensity toward one or the other pathway are nitric oxide (NO) and the release of inhibitory enzymes of the arachidonic acid pathway such as cytochrome P450 (CYP) ω-hydroxylase, CYP epoxygenase, and cyclooxygenase-1 (Blanco et al., 2008; Koehler et al., 2009). However, additional astrocytic mechanisms that are not dependent on astrocytic Ca^{2+} signaling such as those involved in the energy-metabolism-dependent signals (e.g., glutamate transport) are also likely involved (Haydon and Carmignoto, 2006). All of these data strongly suggest a primary role for astrocytes in the regulation of cerebral blood flow during neuronal activation; however, additional studies are required to fully elucidate the number of complex mechanisms that regulate this critical process.

A word of caution must be made regarding recent studies utilizing *in vivo* and *in vitro* preparations to study the regulation of cerebral blood flow by glia (recently reviewed elsewhere, but see Haydon and Carmignoto, 2006; Iadecola and Nedergaard, 2007). Some of the studies have shown changes in vascular diameter that are consistent with post-arterial (perhaps capillary?) structures. Although these changes in diameter (and in intraluminal flow) may account for astrocyte-mediated regulation of flow, it is also possible that under conditions of electrical stimulation such as those used to evoke the release of vasoactive mediators by glia, ionic shifts cause osmotic changes that translate to changes in vascular shape. This phenomenon, if reproduced, may hold significant relevance in pathologies where intracranial pressure or altered extracellular space ion composition causes reduced perfusion and venous return, contributing to ischemic-like pathology.

REGULATION OF CEREBRAL BLOOD FLOW AND BLOOD–BRAIN BARRIER INTEGRITY

From the studies noted above, it is clear that both astrocytes and neurons may release vasoactive substances in response to electrical activity. The mediators involved are all products of synaptic transmission and include ions (protons and potassium; see Table 2.1), metabolic intermediates (adenosine), and neurotransmitters (glutamate). It is important to remember that these signaling molecules are functional only under optimal conditions (i.e., when their levels are regulated to permit subsequent signaling). In many ways, these conditions resemble the "refractory period" that follows the generation of an action potential. If, as in many neurological disorders, the BBB is breached (Grant and Janigro, 2004; Krizanac-Bengez et al., 2004; Oby and Janigro, 2006; Zlokovic, 2008), the reestablishment of a baseline level becomes problematic. For example, the concentration of potassium will increase and the levels of adenosine will decrease after blood–brain barrier disruption. Thus, further release of potassium by neurons may result in a vasoconstriction, which is the opposite of what the system is supposed to do under physiological conditions.

These considerations lead to the important and recently therapeutically exploited strategy of BBB repair to treat neurological diseases (Granata et al., 2008). The rationale for these studies, and similar rodent model experiments (Fabene et al., 2007, 2008), was amelioration of BBB function, but the fact that endothelial repair will also improve brain perfusion must be taken into account. By contrast, procedures and therapies used to improve cerebral blood flow may positively impact BBB function by restoring metabolic support to glia and endothelial cells.

TABLE 2.1
Ionic Concentrations and Osmolarity in Serum and Brain

	Brain	Serum	Ratio
Total osmolarity (mOsm/L)	295	295	1
Water (%)	99	93	1.06
Glucose (mg/dL)	60	90	0.66
Na (mEq/L)	138	138	1
K (mEq/L)	2.8	4.5	0.62
Ca (mEq/L)	2.1	4.5	0.4
Mg (mEq/L)	0.3	1.7	0.17
Cl (mEq/L)	119	102	1.16
pH	7.33	7.41	0.98

Note: See text for details.

GLIAL CELLS, S100β, AND PERIPHERAL MARKERS OF BLOOD–BRAIN BARRIER DISRUPTION

Although it is uncertain whether an intact BBB is important for the development of neurological diseases, expanding evidence implicates a modulatory role in seizure genesis, multiple sclerosis, and Alzheimer's disease (Engelhardt and Ransohoff, 2005; Man et al., 2007; Marchi et al., 2009; Oby and Janigro, 2006; Vezzani and Granata, 2005; Zlokovic, 2008). One of the problems in BBB research has been the lack of reliable methods to measure BBB integrity (Marchi et al., 2003a, 2004a). Opening of the BBB allows molecules normally present in blood open passage into the CNS; however, this opening, unless it involves specific transporters, works bidirectionally. Proteins normally present in blood are free to diffuse into the CNS; in turn, proteins ordinarily present in high concentrations in the CNS are free to diffuse down concentration gradients into the blood. These peripheral substrates can be detected in the blood to evaluate the permeability characteristics of the BBB at any given time. In recently published review articles, Marchi et al. (2003b, 2004a) discussed the ideal properties of a peripheral marker of BBB disruption. Such proteins should have low or undetectable plasma levels in normal subjects, be normally present in cerebrospinal fluid (CSF), and have a higher normal concentration in the CSF than in plasma. Additionally, the CSF concentration of the protein should increase in response to insult or injury. The protein should be normally blocked by the BBB and exhibit flux across the BBB during barrier disruption. Several proteins, including S100β, neuron-specific enolase (NSE), and glial fibrillary acidic protein (GFAP), have been evaluated for this purpose, but only S100β meets the characteristics of having very low plasma levels with a concentration less than that found in the CSF of normal subjects (see Figure 2.2).

Glial cells secrete a number of important factors for brain development (e.g., migration of neurons), establishing the BBB, and, in the mature brain, ensuring the correct function of the brain cells. Among these, the calcium-binding protein S100β plays an important role. S100β is primarily synthesized by the endfeet process of the astrocytes and plays a fundamental role in the trophism of neurons (Nishiyama et al., 2002; Selinfreund et al., 1991). Historically, increases in S100β levels in serum and CSF have been associated with neuronal damage, thus conferring to S100β the clinical relevance of a marker of neuronal dysfunction. However, because neurological diseases are often accompanied by increased BBB permeability, the markers thought to indicate neuronal damage may in fact indicate BBB defects (Marchi et al., 2003a,b; 2004a). Markers of brain damage include NSE and GFAP. In normal subjects, NSE is more concentrated in brain, and S100β is primarily present in CNS fluids. BBB damage in the absence of neuronal damage would be expected to markedly

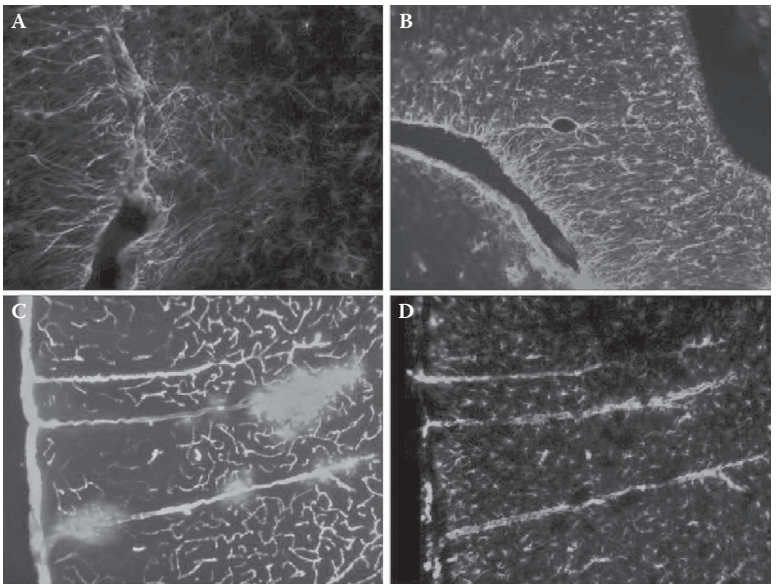

FIGURE 2.2 **(See color insert following page 458.)** Reactive astrocytosis in an animal model of inflammation and seizures: (A, B) treatment with lithium chloride; (C, D) treatment with pilocarpine. Note the enhanced GFAP staining (A, B, D) in specific regions and the leakage of a protein marker (C) in the proximity of gliosis. (From Marchi, N. et al., *Epilepsia*, 48(10), 1934–1946, 2007; Marchi, N. et al., *Neurobiol. Dis.*, 33, 171–271, 2009. With permission.)

increase serum S100β levels while leaving NSE levels unchanged. The fact that serum S100β can be used as a marker of BBB integrity is not necessarily at odds with the notion that S100β is also a marker of brain damage, as both phenomena (BBB failure and brain damage) are temporally and topographically associated (Marchi et al., 2004a).

The plasma levels of S100β are normally a third of those found in the CSF and are nearly undetectable (Janigro et al., 2002). Several diseases cause an elevation in plasma levels of S100β, which can be detected and used for both diagnostic and prognostic purposes, as well as for evaluation of disease progression. Plasma S100β levels increase in cerebral ischemia, with peak levels occurring approximately 3 days after infarction (Buttner et al., 1997; Jonsson et al., 2001). These levels have served as a useful marker of both infarct size and long-term clinical outcome (Dassan et al., 2009; Petzold et al., 2008). Traumatic brain injury has also been shown to increase S100β levels in plasma (Biberthaler et al., 2001a,b), with a positive correlation between the extent of damage after head injury and elevation in plasma S100β.

ROLE OF GLIAL CELLS IN THE DISEASED BRAIN

Over the years, the involvement of glial cells in the pathogenesis and sustenance of CNS diseases has been increasingly demonstrated (De Simoni and Imeri, 1998; De Simoni et al., 2002; Takano et al., 2009; Vezzani et al., 2008a,b). In particular, stroke and epilepsy researchers have focused on the significance of proinflammatory mediators released by glial cells and the possibility of targeting these molecules for therapeutic purposes. In addition to the production of active mediators, glial cells are also involved in chronic changes, either congenital or acquired, including alteration of drug transporters, cytochrome P450 protein expression, and alteration of cell cycle function (Marchi et al., 2004b, 2006; Marroni et al., 2003; Oby et al., 2006).

GLIAL CELLS AND CYTOKINES

In healthy brain, astrocytes control glutamate levels and ion and water homeostasis, release neurotrophic factors, and are a fundamental anatomical–physiological part of the blood–brain barrier (Iadecola and Nedergaard, 2007; Nedergaard et al., 2002). During pathological events, not only are these physiological functions perturbed but reactive phenomena can also take place (Takano et al., 2009). The release of proinflammatory molecules and overexpression of specific inflammation-related receptors are hallmarks of the diseased brain. Whether this reaction is beneficial or detrimental is still a matter of debate. Controversial results have been obtained when modulating proinflammatory responses during CNS pathological events such as ischemic stroke or epileptic seizures (De Simoni and Imeri, 1998; De Simoni et al., 2002; Deng and Poretz, 2003). In addition, our knowledge of how ischemic episodes or seizures affect physiological astrocytic functions (e.g., astrocytic glutamate uptake) is incomplete (Nedergaard et al., 2002).

Glial cells are exquisitely sensitive to neurotransmitters. Astrocytes express neurotransmitter receptors and respond to neuronal activity by increasing cytosolic Ca^{2+} levels according to two distinct modalities—oscillations and propagation (Nedergaard et al., 2002; Newman, 2003). Glial cells are also capable of releasing adenosine triphosphate (ATP) (Rossi et al., 2007). Several modalities of ATP release have been proposed, including channel-mediated release, exocytosis, and P2X7-mediated receptors (Bernardino et al., 2008). Glutamate and ATP/adenosine were demonstrated to be key mediators of astrocytic–neuronal communication. Under normal conditions, ATP released by the astrocytes is converted in the extracellular space by specific nucleotidases into adenosine. Adenosine acts as a neurotransmitter with inhibitory effects on neuronal activity. During a local injury, excessive astrocytic release of ATP activates microglial cells through P2Y12 and P2Y6 receptors. This event leads to the enhancement of phagocytic activity and cytokine release, setting the initial stage of the inflammatory response (Bernardino et al., 2008; Rossi et al., 2007). Adenosine is also a powerful vasodilator (Morii et al., 1986, 1987; Ngai and Winn, 1993).

Recent evidence obtained in animal models of epilepsy and by analysis of surgically resected human epileptic specimens from the temporal lobe has shown that glial cells can reactively produce proinflammatory cytokines in association with ongoing seizure activity. Patients with hippocampal sclerosis display astrocytic activation of the IL-1β system (Vezzani et al., 2008a). Even though the production of proinflammatory molecules has been demonstrated, it is unclear whether blockade of this mechanism could be beneficial in reducing seizure burden, especially considering the etiological and pathophysiological variability inherent among the various epilepsies and even seizure types. Interestingly, acute seizures are prevented by IL-1β antagonists (Marchi et al., 2009), but this effect may be due to intravascular events without involvement of glia. It seems possible, therefore, that acute events are due to the release of IL-1β by white blood cells (or the BBB itself), and chronic conditions characterized by reactive gliosis may involve astrocytes.

GLIAL CELLS, MULTIDRUG TRANSPORTER PROTEINS, AND METABOLIC ENZYMES

Blood–brain barrier expression of multidrug transporter proteins has been extensively studied. In particular, the BBBs of brain tumors and pharmacoresistant epileptic tissues display elevated expression of multidrug-resistant protein 1 (MDR1) (Löscher, 2007; Marchi et al., 2004b, 2006; Marroni et al., 2003). MDR1 was demonstrated to be expressed in astrocytes in brain slices obtained from medically refractory epileptic brain tissues. The question remains whether seizure activity induces MDR expression or MDR is an etiologic factor in epileptogenesis (Marroni et al., 2003). It appears that markers previously associated with chemoresistance of tumor cells are present in epileptic brain, raising the interesting possibility that some overlap exists among tumorigenesis, MDR, and epilepsy. Histopathologically, the most frequent lesions found in drug-resistant patients with

epilepsy include gangliogliomas, and glioneuronal malformations (e.g., hamartias or hamartomas), with a significant overlap between markers of tumorigenesis and epileptogenesis being observed (Marchi et al., 2004b; Marroni et al., 2003).

It was recently proposed that in drug-resistant epilepsy, cellular alterations associated with neoplasms may be present. These include loss of functional expression of p53 in glial cells; thus, in epileptic brain astrocytes, loss of p53 occurs in those cells overexpressing MDR1 proteins (Marchi et al., 2004b; Marroni et al., 2003). In addition to MDR1, epileptic glia express other MDR proteins, including leucine-responsive regulatory protein (Lrp) and multidrug resistance-associated protein 1 (MRP1); these are normally found in tumor cells, suggesting a possible link between drug-resistant epilepsy and low-grade tumors. This evidence suggests that "epileptic" glial cells may have gained a distinct survival advantage. This concept is also supported by evidence showing that blockade of MDR1 function was associated with enhanced drug-induced cytotoxicity. A positive correlation between neuronal and astrocytic expression of MDR1 and a lack of nuclear condensation, a marker of apoptosis and irreversible cell damage, was also observed (Marchi et al., 2004b; Marroni et al., 2003). These studies support the hypothesis that expression of MDR1 in glial cells may protect against toxic xenobiotics or against endogenous compounds that enter the brain under pathological conditions.

Of interest is the relationship existing between MDR1 and P450 metabolic enzymes (CYPs) (Pal and Mitra, 2006). MDR1 and CYPs are under the control of the pregnane X receptor (PXR), a nuclear receptor family regulating a number of enzymes and transporters in mammals. Generally, MDR1, MRPs, and CYPs together constitute a highly efficient barrier for many drugs (Meyer et al., 2007; Pal and Mitra, 2006). CYP glial expression could reinforce the cellular protective effect of MDR1 by metabolizing possible toxins. CYP could also regulate the bioavailability of drugs reaching the brain in a fashion similar to organs such as the gut and liver. CNS expression of CYP1A1, CYP1B1, epoxide hydrolase, and UDP–glucuronosyltransferase was confirmed using rodents models and in human brain tissue (Ghersi-Egea and Strazielle, 2001; Ghersi-Egea et al., 1995, 2001); however, the native pattern of CYP expression in the diseased brain remains elusive.

ELECTRICAL EXCITABILITY HAMPERS GLIOMA GROWTH: SEIZURE AND TUMOR PROLIFERATION

As discussed throughout this chapter, electrical activity exerts profound effects on astrocytic function. In fact, at this stage of modern neuroscience research, it is fair to say that astrocytes are influenced by neuronal activity as much as neighboring neurons. The question that arises is whether effects of electrical field potentials beyond those listed above exist. We noted that cells that sporadically give rise to neoplasms are frequently electrically excitable (cardiac or skeletal muscle, neurons) or surrounded by active cells (pericardium, glia) (Jemal et al., 2004). This has led to investigations into the broad changes in glial cell physiology during or after electrical stimulation. Although we are still far from obtaining a clear picture of the complex mechanisms regulating tumor proliferation, several lines of evidence suggest that ion channels are involved in cancer progression and pathology, including migration and survival (Abdul and Hoosein, 2002; Arcangeli et al., 2009; Fiske et al., 2006; Huang et al., 2009; Le Guennec et al., 2007). Environmental or physiological stimuli that may alter the resting potential of these cells and affect the electrochemical gradient of ions across the cellular membrane are also likely to affect their proliferative state. Correlative *in vivo* data, for example, suggest that conditions of altered electrical activity of the brain, such as during epileptic seizures, may be sufficient to affect the proliferative status of tumor glial cells (Luyken et al., 2003; Majores et al., 2008; Schramm et al., 2004).

The most common electrical event indicating exaggerated brain electrical activity is a seizure. Epileptic seizures are characterized by an abnormal excessive or synchronous neuronal activity in the brain (Fisher et al., 2005). If the hypothesis linking electrical stimulation to proliferation is correct, one may expect that epileptic seizures counter tumor growth. Clinical evidence has shown that

long-term chronic epilepsy that precedes the formation of gliomas significantly decreases mortality (Luyken et al., 2003), suggesting that synchronous, oscillatory, and periodic abnormal electrical activity is not permissive for cell proliferation (Schramm et al., 2004). In addition, long-term epilepsy-associated tumor (LEAT) patients bearing a grade II astrocytoma have a better survival rate than non-LEAT patients; a lower rate of tumor recurrence has also been shown in these patients (Schramm et al., 2004). These findings strongly suggest that the low incidence of tumors or other neoplastic disorders in excitable tissue can be associated with exposure to synchronous, oscillatory, and periodic abnormal electrical activity that may not allow cell proliferation.

A recent report has shown that, at least *in vitro*, low-frequency (comparable to that of seizure activity *in vivo*) and very low-intensity electrical stimulation decreases the proliferative activity of rat glioma and human prostate cancer cells (Cucullo et al., 2005); a mechanism involving potassium channels was shown to be involved. This study provided additional evidence that electrical field potentials and potassium fluxes such as those originating from the neuronal tissue can affect the proliferation of cells that dwell in the proximity of neurons (e.g., glial cells). Although we are not in a position to catalog tumors as channelopathies arising directly from Na^+, K^+, and Ca^{2+} ion channels, several studies have shown that tumors may indeed modulate their functional expression and activity (Bubien et al., 1999, 2004; Olsen et al., 2005; Ross et al., 2007).

Ion channel functions are not limited to excitability and ion homeostasis. In fact, many studies suggest a clear association between the expression of specific classes of ion channels and modulation of different aspects of cellular activity. In *weaver* mice, for example, the mutation in the gene coding for G-protein-activated inwardly rectifying potassium channel subunit 2 (GIRK2) causes the loss of ion selectivity. This leads to the induction of cell death in the external germinal layer of the cerebellum and alterations in neurite extension and cell migration (Migheli et al., 1999; Patil et al., 1995). Ion channels also have a major role in the cellular regulation of proliferative activity comparable to glial cells.

Recent studies have shown that the inhibition of K_{ir} channels reactivates the proliferative activity in quiescent glia (MacFarlane and Sontheimer, 2000). This suggests that the downregulation of K_{ir} may promote cell cycle progression, while its premature expression or overexpression is associated with cell cycle arrest in G1/G0. This regulatory activity works both ways, however, such that the expression level of a specific ion channel is also regulated by the proliferative state in which the cell resides at a particular time; for example, the arrest of spinal-cord astrocytic growth at defined stages of the cell cycle causes significant changes in the expression of voltage-activated Na^+ and K^+ currents (MacFarlane and Sontheimer, 2000). Different types of ion channels, such as K^+, Cl^-, and Ca^{2+}, appear involved in normal cell cycling in different cell types. It is commonly held that the activation of Ca^{2+} channels is often related to increases in intracellular Ca^{2+} ($[Ca^{2+}]_i$) during cell cycle progression, whereas the inhibition of Cl^- or K^+ channels often blocks the cell cycle without affecting cell viability.

K^+ channels represent a large and heterogeneous family with over 80 genes encoding for various proteins that control membrane potential. Their role in the regulation of cell cycle progression has been widely studied, especially for their involvement in tumor cell proliferation (Brevet et al., 2008). Thus, K^+ channels have become potential targets for the development of novel therapeutic treatments (Villalonga et al., 2007); for example, with respect to tumor growth, voltage-gated and ATP-sensitive K^+ channels have been involved with the cell proliferation of gliomas (Lee et al., 1994; Preussat et al., 2003). More recently, experiments performed *in vitro* have highlighted a previously unrecognized role for GIRK2 (or $K_{ir}3.2$) in human glia (Cucullo et al., 2005). In an experiment involving electrical stimulation, the investigators found a direct causal role between the expression of this potassium channel and the antiproliferative effect of low-frequency, ultra-low-intensity alternating electric current (Cucullo et al., 2005). Large-conductance Ca^{2+}-activated K^+ currents have also been characterized in glioblastoma cell lines (Olsen et al., 2005), and a mislocalization of K_{ir} channels primarily into the nucleus has been described in malignant glioma cell lines (Lee et al., 1994).

Taken together, these findings strengthen the importance of potassium channels in tumor differentiation and proliferation and explain their current status as major pharmaceutical targets for the development of novel antineoplastic and antiepileptic agents (Arcangeli et al., 2009; Felipe et al., 2006; Le Guennec et al., 2007).

CONCLUSIONS

After decades of neglect, astrocytes have become one the hottest items in neurological research. The major pitfall at this juncture appears to be over-interpretation of results. The data cited above and related to cerebral blood flow regulation by astrocytes, for example, are in partial disagreement with the neuroanatomy that shows a separation of glia and vascular smooth muscle at the Virchow–Robin triad (del Zoppo, 2008). In addition, the caliber of vessels involved suggests recruitment of capillary cells, which by definition are devoid of vascular smooth muscle. Are pericytes involved, or is the observed change due to shifting osmotic gradients? Further studies will clarify this. The results linking cell cycle to electrical stimulation may similarly propose mechanisms that bear little significance to tumor treatment. It is hoped that *in vivo* experiments will shed light on this. It is also possible that the link among cell cycle, glia, and ion channels goes beyond cancer; for example, a loss of K_{ir} has been reported in a variety of neurological disorders, such as epilepsy (astrocytes, but perhaps other cell types) (Bordey and Sontheimer, 1998; Bordey et al., 2001) and ischemia (vascular smooth muscle cells) (Marrelli et al., 1998). Finally, the discovery of additional noninvasive peripheral markers—including serum protein of astrocytic origin other than S100β—would greatly enhance the clinical significance of these laboratory studies.

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3 Metabolic Regulation of Seizures and Epileptogenesis

Manisha Patel

CONTENTS

INTRODUCTION

Metabolism is defined as an organized set of chemical reactions comprising metabolic pathways that are used by living organisms to transduce and store energy and thereby maintain life. Metabolic processes are tightly regulated by enzyme levels, their catalytic activities, and the availability of substrates. Metabolic processes are critical in maintaining neuronal excitability; therefore, synchronized neuronal firing associated with seizure activity *per se* represents a "metabolic challenge." Acute seizure activity associated with status epilepticus (SE) and chronic seizures constitute metabolic irregularities. Metabolism operates in discrete yet interrelated biochemical circuits with the central goal of transducing energy as well as information via signal transduction. Metabolic pathways have remarkable adaptability, capacity, and flexibility that allow maintenance of homeostasis and therefore make metabolic disorders often difficult to discern.

The role of metabolism is recognized in many human conditions such as diabetes, cancer, and various neurodegenerative diseases such as Parkinson's disease, Huntington's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (ALS) (Beal, 2003; Hall et al., 1998; Halliwell, 1992; Schapira et al., 1993). Common mechanisms that form the metabolic hypothesis in these neurodegenerative diseases include aging, metabolic impairment, mitochondrial dysfunction, oxidative damage, excitotoxicity, and selective vulnerability (Beal, 1998, 2000, 2003). Each of these mechanisms is thought to contribute to the pathogenesis of temporal lobe epilepsy (TLE). In fact, the importance of metabolism in the epilepsies has been appreciated for over a century and is based on strong neurochemical, imaging, and physiological data.

METABOLIC REGULATION OF EPILEPTIC BRAIN DAMAGE

The role of cell loss in epilepsy has been recognized for over a century. Important confirmation of the occurrence of neuronal loss in epilepsy came from work by Meldrum and colleagues (1983, 2002), who showed that seizures *per se* but not associated systemic complications resulted in hippocampal

lesions. Several key studies have confirmed this (Olney, 1986; Slovitor, 1983) and further described lesions in multiple brain areas by seizure activity (Ben-Ari et al., 1981; Lothman, 1990; Nadler, 1979; Olney et al., 1983). The recognition that seizure-related brain damage was pathologically similar to excitotoxicity (Olney, 1986) is in itself evidence for the metabolic hypothesis, given the early observation that pathological and mechanistic features of glutamate-induced excitotoxic cell death involve energy-dependent processes (Henneberry et al., 1989a,b; Olney et al., 1983). The role of mitochondrial bioenergetics is now widely recognized in excitotoxic cell death (Nicholls and Budd, 2000).

In addition to excitotoxic mechanisms, apoptotic pathways are also activated by prolonged seizures in human and experimental epilepsy (Henshall and Murphy, 2008). The inherent energy dependence and mitochondrial involvement of apoptotic pathways—particularly the intrinsic pathway—provide yet another mechanism through which metabolic factors influence epileptic brain damage. Additionally, the intricacy of apoptotic signaling provides numerous therapeutic targets for controlling seizure-induced brain damage.

Although cell loss can occur following prolonged seizure activity, recent studies suggest that it is not necessary for metabolic dysfunction (Cohen-Gadol et al., 2004; Hugg et al., 1996; Vielhaber et al., 2008). In fact, metabolic impairment is prevalent in surviving neuronal subfields as well as non-neuronal cells in human TLE (Vielhaber et al., 2008). This suggests that neuronal loss *per se* is not the underlying cause of metabolic dysfunction in the epileptic brain. Because metabolic impairment can contribute to seizure-induced neuronal death and can occur independent of neuronal death, therapeutic targeting of metabolism may provide dual benefits.

ROLE OF GLYCOLYSIS IN SEIZURE CONTROL

Oxidation of glucose is the major source of cellular energy in the brain, although the brain can efficiently utilize alternative fuels such as ketones and fatty acids. Seizure activity produces dramatic increases in glucose uptake and metabolism. This increase is unparalleled by most other conditions. Cerebral blood flow increases to match this hypermetabolism. The increased rate of glycolysis exceeds pyruvate utilization by pyruvate dehydrogenase, resulting in an increased lactate buildup. Several intriguing studies have shown a close link between seizure activity and high glucose concentrations, as well as its utilization via glycolysis. First, high glucose concentrations in the blood exacerbate seizures (Schwechter et al., 2003), whereas moderately low glucose levels have the opposite effect (Greene et al., 2001). Second, the collective information obtained from human imaging studies suggests that *hypermetabolism* occurs in human epileptic foci during ictal phases (seizure episodes) and *hypometabolism* during interictal phases. Third, 2-deoxyglucose (2-DG), a nonmetabolizable sugar, or bypassing glycolysis via fructose-1,6-bisphosphate has been shown to exert anticonvulsant effects *in vivo* (Garriga-Canut et al., 2006; Lian et al., 2007). Finally, the switching of fuels from glucose to ketones (thus bypassing glycolysis) with the ketogenic diet also results in an anticonvulsant effect (Melo et al., 2006).

Collectively, these studies link seizure activity with high glucose concentrations. Although recent progress has been made regarding the potential mechanisms by which 2-DG and the ketogenic diet exert an anticonvulsant effect, the precise mechanism by which glycolysis exacerbates seizures remains to be determined. Because glycolysis provides carbon sources for mitochondrial energy production, mitochondrial mechanisms may ultimately underlie the damaging effect of glycolysis on seizure activity.

ROLE OF MITOCHONDRIA IN EPILEPSY

The involvement of mitochondria in normal and excessive neuronal excitability is obvious given the bioenergetic requirements of the process. Mitochondria burn dietary calories with oxygen to produce work and heat. A byproduct of this process is the production of reactive oxygen species (ROS),

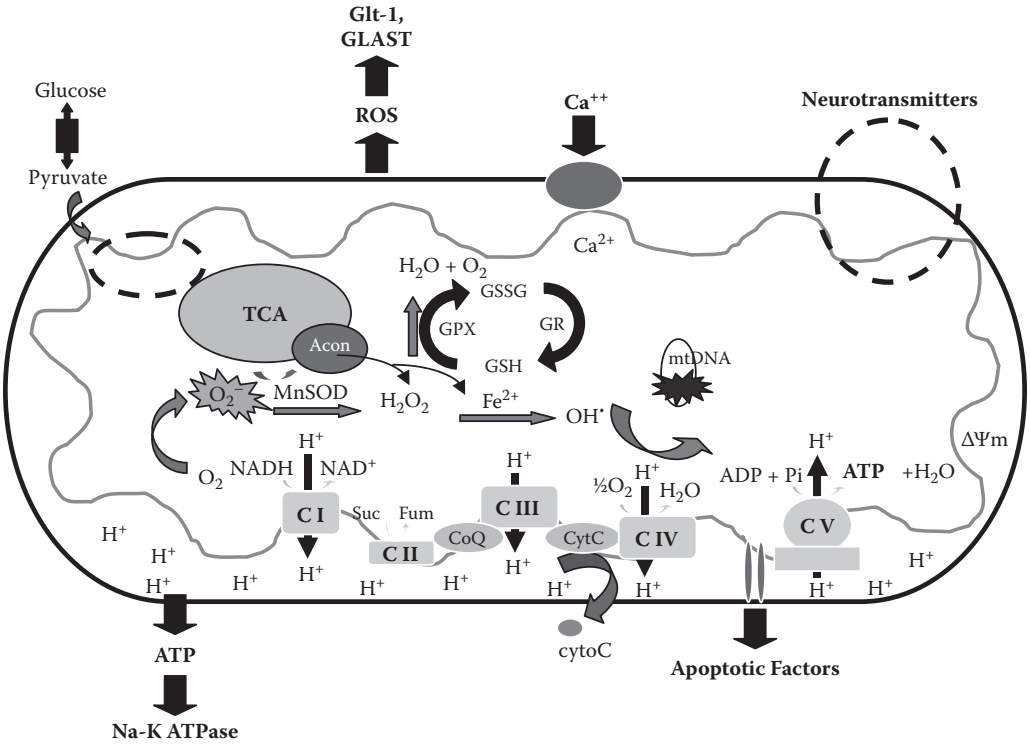

FIGURE 3.1 **(See color insert following page 458.)** Overview of important mitochondrial functions relevant to neuronal excitability.

which can damage cellular DNA, proteins, and lipids. In addition to adenosine triphosphate (ATP) and ROS production, several other mitochondrial functions, such as fatty acid biosynthesis, amino acid cycling, neurotransmitter biosynthesis, and ionic homeostasis (particularly calcium buffering), could also impact neuronal excitability (Figure 3.1). Mitochondrial decline and mitochondrial DNA (mtDNA) damage are thought to play a central role in the etiology of age-related metabolic and degenerative diseases (Wallace, 2005). ROS are thought to play a key role in this process by influencing the extent of mitochondrial decline. The increased incidence of epilepsy with advancing age as well as the progressive nature of some acquired epilepsies (e.g., TLE) strongly suggest that mitochondrial decline may be a critical mechanism in its etiology, much like more commonly understood neurodegenerative diseases.

Imaging Studies of Human and Animal Tissue

Human imaging studies have by far provided the most compelling evidence for mitochondrial dysfunction in epilepsy. A detailed review of human imaging studies and neurotransmitter changes can be found in Pan et al. (2008). Collectively, these imaging studies demonstrate dramatic metabolic and bioenergetic changes based on measurement of glucose, oxygen, and mitochondrial *N*-acetylaspartate (NAA) levels. The strongest evidence for mitochondrial involvement in mesial temporal lobe epilepsy comes from measurement of NAA using magnetic resonance spectroscopy (Cendes et al., 1994; Hetherington et al., 1995; Petroff et al., 2002). The loss of NAA, which is known to be specifically synthesized by neuronal mitochondria, has been shown by various groups to be subregion specific, reversible by surgical or pharmacological intervention, and unrelated to neuronal death (Clark, 1998; Vielhaber et al., 2003). The collective interpretation of imaging studies

demonstrating the decrease in NAA in human TLE points toward mitochondrial dysfunction as the cause rather than neuronal loss (Vielhaber et al., 2008). Severe metabolic dysfunction characterized by biphasic abnormal nicotinamide adenine dinucleotide phosphate (NADPH) fluorescence transients and changes in mitochondrial membrane potential ($\Delta\Psi$) have been observed in *ex vivo* preparations from both chronically epileptic rats and human subjects (Kovacs et al., 2002).

Mitochondrial Dysfunction: A Consequence of Acute and Chronic Seizures

An important combustion byproduct of mitochondrial metabolism is the production of ROS. While abundant and overlapping endogenous antioxidants exist to overcome normal cellular ROS production, excessive production of ROS can overwhelm antioxidant defenses, resulting in oxidation of vulnerable cellular targets. Using surrogate markers of target oxidation in the kainic acid model, work from this laboratory has demonstrated that SE can oxidatively damage mtDNA and susceptible mitochondrial proteins and increase cellular lipid peroxidation (Jarrett et al., 2008a; Liang and Patel, 2006; Liang et al., 2000; Patel et al., 2001, 2008). The increased vulnerability of mtDNA to seizure-induced damage in comparison to nuclear DNA is consistent with the appearance of 8-hydroxy-2-deoxyguanosine (8-OHdG), the oxidative base lesion in mitochondrial (but not nuclear) fractions, and a greater frequency of mtDNA lesions. Expression of mitochondrial base excision repair enzymes (8-oxoguanine glycosylase and DNA polymerase gamma), reflected by increased mtDNA repair capacity, occurs shortly following an episode of SE; however, mitochondrial ROS production and mtDNA damage emerge again during recurrent seizures associated with the chronic phase of epilepsy concomitant with the failure of repair processes. In addition to being an acute consequence of SE, mitochondrial ROS production resurfaces during chronic epilepsy, suggesting that ROS formation and mtDNA damage could contribute to epileptogenesis (Jarrett et al., 2008a).

Whether and how mitochondrial ROS and the resulting dysfunction lower seizure threshold and contribute to epileptogenesis remain unknown. Assessment of mitochondrial dysfunction several weeks after SE was also addressed in pilocarpine-induced SE (Kann et al., 2005; Kudin et al., 2002). A prominent finding from these studies is the decrease in complex I activity of the electron transport chain (ETC), accompanied by lowered mitochondrial membrane potential measured by rhodamine-123 fluorescence in the CA1 and CA3 subfields of the hippocampus, perhaps due to a decreased mitochondrial DNA copy number that results in downregulation of oxidative phosphorylation (OXPHOS) enzymes encoded by mtDNA.

Together, these independent studies in two distinct animal models suggest that the acute effects of SE (i.e., increased mitochondrial oxidative stress) may result over time in oxidative damage to mtDNA (as suggested by increased levels of 8-OHdG) and decreased expression of mitochondrially encoded proteins required for the functioning of the ETC. Seizure-induced accumulation of oxidative mitochondrial DNA lesions and resultant somatic mtDNA mutations could, over a period of time, render the brain more susceptible to subsequent epileptic seizures, particularly in the context of advancing age. Human studies have confirmed at least some aspects of mitochondrial dysfunction seen in animal models. Complex I deficiency, which is a leading cause of increased superoxide production, and inhibition of aconitase, a marker of ROS, have been observed in the seizure foci and surviving CA3 cell layer, respectively, in tissue from TLE patients (Vielhaber et al., 2008). The increase in nitric oxide (NO) after seizure activity can either directly or indirectly (via reaction with superoxide and peroxynitrite formation) modulate the activity of the ETC complexes and thereby contribute to the metabolic changes associated with epilepsy.

Mechanistic studies assessing the role of mitochondrial functions on neuronal excitability have been conducted by measuring seizure-like events (SLEs) in hippocampal explant cultures. The SLEs are usually generated by a combination of electrical stimulation of the axons of the granule cells (i.e., the mossy fibers) and lowering the Mg^{2+} concentration in the bathing media (0 Mg^{2+}) to relieve the voltage-dependent Mg^{2+} blockade of the *N*-methyl-D-aspartic acid (NMDA) receptors. The SLEs are associated with increased mitochondrial calcium accumulation, mitochondrial depolarization,

decreased NADPH autofluorescence, and superoxide generation. Superoxide production measured by dihydroethidium progressively increases during and with each consecutive SLE, along with increases of both cytosolic and mitochondrial calcium, thus providing a link between mitochondrial calcium, free-radical production, and neuronal death.

The mechanism by which seizure activity increases mitochondrial ROS remains incompletely understood. Although changes in mitochondrial calcium levels may explain the increased ROS formation, a discrepancy exists between ROS production due to seizure-induced changes in the mitochondrial membrane potential and calcium elevation. Seizure-induced mitochondrial ROS production occurs in the face of a loss of mitochondrial membrane potential, which is usually associated with decreased ROS production via the ETC complexes. This suggests that alternative mechanisms of ROS production may occur following seizures, such as inactivation of the tricarboxylic acid (TCA)-cycle enzyme aconitase which can release hydrogen peroxide.

Mitochondrial Dysfunction and Neuronal Excitability

Although mitochondrial dysfunction is an acute and chronic consequence of epileptic seizures, whether mitochondrial dysfunction can be a causative factor in epileptogenesis is unknown. The precedence for mitochondrial dysfunction initiating epilepsy has already been established from our knowledge regarding the molecular basis of certain inherited epilepsies (i.e., the mitochondrial encephalopathies). The most prominent example of mitochondrial dysfunction causing epilepsy is the occurrence of epilepsy in mitochondrial disorders due to mutations in mitochondrial DNA or nuclear DNA (DiMauro et al., 1999). Myoclonic epilepsy with ragged red fibers (MERRF) is a syndrome wherein a single mutation of the mitochondrially encoded tRNALys results in a disorder consisting of myoclonic epilepsy and a characteristic myopathy with ragged red fibers (Shoffner et al., 1990; Wallace et al., 1988). Defects in complex I and complex IV of mitochondrial OXPHOS are the leading mechanisms by which this mitochondrial gene mutation produces MERRF (Wallace et al., 1988).

Several normal functions of mitochondria, ranging from its bioenergetic functions to metabolic functions, can impact neuronal excitability. These include cellular ATP production, ROS formation, synthesis and metabolism of neurotransmitters, fatty acid oxidation, calcium homeostasis, and control of apoptotic/necrotic cell death. These vital functions are closely interrelated, but which of these factors contributes to the seizures associated with mitochondrial dysfunction remains unclear. Although mitochondrial encephalopathies due to genetic causes are rare, they may provide important lessons regarding the mechanisms underlying acquired epilepsy, such as temporal lobe epilepsy. By contrast with the gene defects of mitochondrial proteins, acquired epilepsy may arise due to chronic mitochondrial dysfunction that results in damage to the mitochondrial genome or mitochondrial bioenergetics. Factors that can promote acute and chronic mitochondrial dysfunction, which may in turn damage the mitochondrial genome, include hypoxia, trauma, and aging itself, as well as comorbid neuronal diseases such as stroke and Alzheimer's disease. ROS may be an important weapon triggered by each of these conditions that is capable of inducing direct damage to the mitochondrial genome. This can result in decreased expression of mtDNA-encoded OXPHOS subunits, known to result in epileptic seizures.

Another prominent target that links mitochondrial dysfunction and resultant ATP depletion with increased neuronal excitability is the plasma-membrane-bound sodium–potassium ATPase. Inhibition of the sodium–potassium ATPase has been shown to result in seizure activity (Grisar et al., 1992), and a defect in the $\alpha 3$ isoform of this enzyme renders the sodium pump dysfunctional and contributes to hyperexcitability and seizures (Clapcote et al., 2009). Other targets of ROS that may increase excitability are glutamate transporter 1 (GLT-1) and glutamate aspartate transporter (GLAST), which are known to be redox sensitive (Trotti et al., 1998) and play a crucial role in maintaining low levels of synaptic glutamate. Age-dependent seizures occurring in the context of chronic mitochondrial oxidative stress in Sod2(–/+) mice are accompanied by decreases in the

hippocampal expression of the glial glutamate transporters GLT-1 and GLAST, which may explain their increased vulnerability to epileptic seizures (Liang and Patel, 2004). This is consistent with increased hippocampal extracellular glutamate levels that have been found in epileptic patients (During and Spencer, 1993).

METABOLIC INTERVENTIONS

Anticonvulsant drugs remain frontline therapies for controlling epilepsies and serve to decrease neuronal excitability. Metabolic approaches may provide anticonvulsant effects in humans as well, but this has yet to be fully validated. A recent investigational drug screening approach is to search for antiepileptogenic, rather than antiepileptic, drugs that would target underlying processes that lead to the development of epilepsy. Antiepileptogenic therapies aimed at mitochondrial bioenergetics and oxidative stress pathways have been largely limited to animal studies. Clinical trials of vitamin E as an add-on therapy for refractory epilepsy have yielded conflicting results, with largely failed attempts to reduce epileptic seizures in pediatric patients. Creatine, an endogenous guanidine, functions with phosphocreatine and a mitochondrial form of creatine kinase as a spatial energy buffer between the cytosolic and mitochondrial compartments. Creatine has been shown to be protective in animal models of central nervous system (CNS) injury, including trauma, ischemia, the 3-nitropropionic acid model, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism, and ALS (Klivenyi et al., 2003; Matthews et al., 1998, 1999). Creatine supplementation has been effective in reducing hypoxia-induced seizures in both rats and rabbits (Holtzman et al., 1998, 1999).

The clinical management of intractable epilepsies with strategies such as caloric restriction or modification (e.g., through the use of a ketogenic diet) supports a role for mitochondrial bioenergetics in the protective effects. The ketogenic diet has been shown to produce mitochondrial biogenesis and bioenergetically efficient mitochondria, to increase mitochondrial glutathione levels, and to lower levels of ROS (Bough et al., 2006; Jarrett et al., 2008b; Sullivan et al., 2004). The lower levels of ROS have been shown to occur due to upregulation of the mitochondrial uncoupling protein 2 (UCP2) in mice fed a ketogenic diet (Sullivan et al., 2004). In these experimental studies, seizure-induced brain damage and subsequent epilepsy could be inhibited by caloric restriction or a ketogenic diet (Greene et al., 2001; Todorova et al., 2000).

Diet modification by ketogenic diet or caloric restriction represents a nonpharmacologic strategy that decreases seizure frequency in the epileptic EL mouse (Todorova et al., 2000). Interestingly, these paradigms are also known to limit free-radical formation. Consistent with this effect, recent studies in our laboratory have shown an upregulation of glutathione biosynthesis in animals fed a ketogenic diet (Jarrett et al., 2008b). In this study, it was demonstrated that the ketogenic diet specifically increases mitochondrial glutathione levels, stimulates *de novo* glutathione (GSH) biosynthesis, and improves mitochondrial redox status in the hippocampus, resulting in decreased mitochondrial ROS production and protection of mtDNA (Jarrett et al., 2008b). Thus, increased mitochondrial GSH may represent a possible candidate mechanism underlying the protection afforded by the ketogenic diet. In summary, the recent resurgence of research on the mechanisms of the ketogenic diet may identify novel neuroprotective and anticonvulsant therapeutic targets.

Several newer antiepileptic drugs such as zonisamide, topiramate, and levetiracetam inhibit mitochondrial dysfunction and possess antioxidant properties, raising the possibility that inhibition of mitochondrial dysfunction may in part underlie their actions. Oxidative stress and neuronal damage induced by kainate can be ameliorated by at least two types of superoxide dismutase (SOD) mimetics: the manganese porphyrin MnTBAP and the salen–manganese compound EUK-134. However, acute administration of catalytic antioxidants does not alter chemoconvulsant-induced behavioral seizure severity. Previous generations of manganese porphyrins (e.g., MnTBAP) showed limited ability to cross the blood–brain barrier (BBB), which necessitated intracerebral administration, and it remains to be determined if newer classes that are BBB permeable, orally active, and validated

in animal models of neurodegeneration modulate seizure-induced injury or epileptogenesis. Other compounds with antioxidant properties that inhibit seizure-induced brain injury include the hormone melatonin, a nitrone spin trap, and vitamin C.

It should be noted that because ROS play an important physiological role in cell signaling, their removal with antioxidants may have deleterious consequences on cellular functions during chronic administration. Although iron chelators are poorly tolerated *in vivo*, chelation of the mitochondrial pool may be another avenue for therapeutic intervention. One promising example is the use of *N*,*N*′-*bis*(2-hydroxybenzyl) ethylenediamine-*N*,*N*′-diacetic acid (HBED), a lipophilic iron chelator that penetrates mitochondria following *in vivo* administration and inhibits seizure-induced hippocampal injury (Liang et al., 2008).

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4 Brain Inflammation and Epilepsy

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CONTENTS

INTRODUCTION

Inflammation includes a variety of protective processes that have evolved to activate a defensive attack against noxious stimuli, thus representing an important endogenous homeostatic mechanism of the organism. Usually, the outcome of the inflammatory program is a rapid repair of tissue damage; however, if these processes are not properly controlled in timing and extent, then inflammation becomes deleterious, thus leading to permanent tissue damage and cellular dysfunction, as suggested to occur in chronic neurodegenerative disorders and in epilepsy.

This chapter provides a brief overview of the experimental and clinical evidence linking brain inflammation to epilepsy. We describe three groups of inflammatory mediators—namely, cytokines, the complement system, and cyclooxygenase-2 (COX-2). Their expression is increased in rodent and human epileptogenic tissue, such as in temporal lobe epilepsy and malformations of cortical development that do not feature a typical inflammatory pathophysiology. Pharmacological attempts have been made in experimental models to understand their functional role in seizure activity, epileptogenesis, and seizure-induced neuronal cell death. Current knowledge suggests an involvement of these specific inflammatory pathways in the pathogenesis of seizures, thus highlighting new potential therapeutic strategies. In this chapter, we first describe changes in the expression of cytokines, the complement system, and COX-2 in epileptogenic tissue of experimental models and humans. We next discuss the pharmacological data addressing the functional consequences of brain expression of these inflammatory mediators on seizures, epileptogenesis, and cell loss. Finally, recent evidence describing the mechanisms by which brain inflammation can alter neuronal circuit excitability and seizure activity is reported.

SEIZURE-INDUCED EXPRESSION OF INFLAMMATORY MEDIATORS

Experimental Models

Proinflammatory Cytokines

A novel concept emerging from the literature is that seizure activity induced in rodents increases the production of various inflammatory molecules in the brain. In particular, a rapid inflammatory response has been detected in response to seizures induced by chemoconvulsant application, electrical stimulation, or kindling in brain areas recruited in the generation and spread of seizures (De Simoni et al., 2000; Gorter et al., 2006; Plata-Salaman et al., 2000; Shinoda et al., 2003; Vezzani et al., 1999). This response includes a rapid increase of proinflammatory cytokines, such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) prominently in glia, and also involves endothelial cells and neurons (De Simoni et al., 2000; Ravizza et al., 2008a; Vezzani et al., 1999). This phenomenon is followed by a cascade of downstream inflammatory events, such as upregulation of Toll-like receptors (TLRs), activation of NFκB, chemokine production, complement system activation, and increased expression of adhesion molecules (Aronica et al., 2007; Fabene et al., 2008; Librizzi et al., 2007; Turrin and Rivest, 2004; Vezzani and Granata, 2005; Vezzani et al., 2008). Using experimental models of status epilepticus (SE) in which epileptogenesis is triggered and spontaneous seizures occur, we analyzed the temporal evolution of inflammatory changes in the brain. We studied the immunohistochemical pattern of IL-1β and its signaling receptor IL-1 receptor type 1 (IL-1R1) as prototypical markers of inflammation (Figure 4.1).

IL-1β is barely detectable in healthy brain, but during the acute phases of SE it is highly expressed by microglia (Figure 4.1A) and reactive parenchymal (Figure 4.1B) and perivascular astrocytes (Ravizza et al., 2008a). IL-1β upregulation persists during epileptogenesis (Figure 4.1C), in the absence of ongoing seizure activity, and in chronic epileptic tissue characterized by spontaneous and recurrent seizures (Figure 4.1D) (Ravizza et al., 2008a). IL-1R1 upregulation involves both neurons (Figure 4.1E–G) and astrocytes (Figure 4.1H) (Ravizza and Vezzani, 2006; Ravizza et al., 2008a), suggesting that this cytokine released by glia exerts both autocrine and paracrine actions. IL-1β and IL-1R1 are highly expressed during epileptogenesis, both in the astrocytic endfeet impinging on brain microvasculature and in endothelial cells of the blood–brain barrier (BBB). These events occur in areas of BBB leakage and neuronal damage (Ravizza et al., 2008a), thus suggesting that inflammation may be responsible for these pathological changes.

The production of proinflammatory molecules is usually accompanied by the concomitant synthesis of antiinflammatory mediators to modulate the inflammatory response and avoid the occurrence of deleterious effects. In this respect, IL-1 receptor antagonist (IL-1Ra), a naturally occurring IL-1β antagonist, also increases during seizures (Figure 4.1I). IL-1Ra is induced in the brain several