

Epileptiform activity in the limbic system

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1. ABSTRACT

Mesial temporal lobe epilepsy (MTLE) is a common neurological disorder characterized by hyperexcitability of limbic structures. Studies in epileptic patients and animal models of MTLE indicate that epileptiform activity arise primarily from limbic areas (e.g. hippocampus) with secondary propagation to cortical areas. A wealth of evidence indicates that epileptiform activity is associated with complex patterns in the expression and function of ion channels, receptors and transporters. Accordingly, several studies portrait MTLE as a post-transcriptional acquired channelopathy. The present review describes the most common features of epileptiform activity emerging from animal models of limbic epileptogenesis and critically discusses the supporting evidence that MTLE is a complex acquired channelopathy.

2. INTRODUCTION

Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures (1). These seizures are transient signs and/or symptoms of abnormal, excessive or synchronous neuronal activity (abnormal electrical discharges) in the brain (2,3). Epilepsy is a major health problem that affects 2% of the general population posing tremendous challenges to modern medicine. Although a large number of antiepileptic drugs (AEDs) that suppress or prevent seizures are now available, about 30–40% of the patients, children as well as adults, remain resistant to drug treatment. Accordingly, pharmacoresistance is present in about 30% of patients suffering mesial temporal lobe epilepsy (MTLE), one of the most common causes of acquired partial epilepsy, defined by the occurrence of either simple partial or complex

partial seizures (4). These patients become candidates for surgical resection of the epileptogenic area, in most cases located in the limbic system (*i.e.* hippocampus and parahippocampal regions) (5). Accordingly, ameliorating epileptogenesis and solving the problem of medical intractability are unmet medical challenges. "Epileptic phenotypes" at neuronal and network levels may ultimately emerge from small, but critical alterations in ion channels or receptors. During the process of epileptogenesis, seizure-related functional and structural reorganizations of neuronal circuits lead to a form of maladaptive neuroplasticity characterized by hyperexcitability of glutamatergic neurons and defective inhibition (6,7).

Interictal spikes are periodic, very brief bursts of neuronal activity that are observed in the electroencephalogram of patients with chronic epilepsy (8). Classical studies indicate that the electrophysiological correlate of interictal spikes recorded by surface and deep brain electrodes is a paroxysmal depolarization shift (PDS) of the membrane potential when recording of electrical activity is performed simultaneously with intracellular microelectrodes in neurons located in the epileptogenic area (9,10). Bursts of action potentials at high frequency are triggered above membrane threshold during the PDS phenomena, which are associated with polyspiking "epileptiform" activity of network neurons located in the same area (10). Different degrees of epileptiform activity are relatively preserved across different epilepsy models and have been considered a sign of hyperexcitability. Although the electrophysiological signature of hyperexcitability follows this stereotyped pattern of electrical activity, the mechanisms that transform relatively normal neuronal networks into "epileptogenic" areas are quite complex, and remain not completely understood. In the last decades, investigators have focused on determining how abnormalities in intrinsic properties and synaptic functions contribute to epileptogenesis in an effort to develop comprehensive therapies for the pharmacological management of epilepsy. Indeed, molecular and functional aberrations of ion channels and receptors ("channelopathies") at somatic and dendritic locations have been recently reported as underlying pathogenic mechanisms leading to neuronal hyperexcitability and abnormal postsynaptic integration in experimental and human epilepsy (11,12). These seizure-related abnormalities in the expression of ion channels are considered a key determinant of epileptogenesis and medical intractability. Epileptogenesis refers to the complex molecular and cellular pro-epileptic transformations that are triggered in the normal brain tissue by the initial brain insult (*i.e.*, *status epilepticus*, traumatic brain injury, *encephalitis* and *meningitis*, *etc.*) leading to increased cellular and network excitability and the emergence of recurrent spontaneous seizures (13). As indicated by Jacobs *et al* (2009), the concept of a "latent" or "silent" period in humans and animal models of MTL has been useful to describe this transition. Moreover, epileptogenesis may also develop during the progression of the disease triggered by recurrent seizures and underlying molecular and cellular disturbances. Hence, epileptogenesis is considered to include both the initial development of the

epileptic state and the seizure-related abnormalities during the progression of the disease. Elucidation of the precise mechanisms responsible for epileptogenesis may provide a comprehensive strategy to develop preventive measures to arrest pro-epileptic changes and to treat epilepsy.

Early or preventive treatment remains a polemic issue in pharmacotherapy of epilepsy. In this regard, some of the major challenges of epileptology are (a) to reliably determine whether a patient will develop epilepsy after suffering a brain insult (*i.e.*, *status epilepticus*, traumatic brain injury) and (b) to identify whether a patient suffering epilepsy will develop refractory epilepsy. Meeting these challenges is a high priority because early initiation of aggressive therapy has been proposed as a rational approach that may improve outcome and overall quality of life (14,15). These challenges are associated with the need of effective biomarkers of the disease (e.g., biomarkers of intractability or epileptogenesis) and a comprehensive assessment of potential "acquired" and "genetic" risk factors to identify patients in an early stage of the disease or at high risk to progress to pharmacoresistance. There are no biomarkers available to guide neurologists in the identification of these problems with confidence (14-18). In some cases, syndromic classification and clinical evolution of the disease have allowed to predict intractability (14,17). In an attempt to identify patients at risk for medical intractability, Kwan and Brodie (2000) performed a prospective study involving 525 patients (age, 9 to 93 years) who were given a diagnosis, treated, and followed up for 2 to 16 years. These authors concluded that patients who have many seizures before therapy, or who have an inadequate response to initial treatment with antiepileptic drugs are likely to have refractory epilepsy (18). However, it remains unclear which specific subset of molecular, neuroimaging, electrographic and clinical biomarkers has a predictive power of determining intractability.

Surgery for refractory focal epilepsies has been established as a promising treatment option. Technological advances in multimodal neuroimaging (*i.e.* fMRI and PET) and electroclinical evaluation have considerably improved preoperative assessments, safety and outcomes of surgical procedures for the treatment of refractory epilepsy (19). However, the current resolution of neuroimaging tools is not adequate to identify subtle cellular and circuit structural abnormalities underlying seizure generation in some cases of intractable focal epilepsy without major neuroimaging structural correlates (*e.g.* cerebral cortical dysgenesis) (20). Furthermore (21), these methods are unable to detect molecular changes at the level of receptors, ion channels and transporters ("molecular determinants of refractoriness") that may constitute potential biomarkers for early diagnosis of pharmacoresistance. A major advance in this area was the introduction of *in vivo* (^{11}C)flumazenil-positron emission tomography (^{11}C)FMZ-PET, an approach that has allowed the detection of reductions of central benzodiazepine receptors in lesional and perilesional areas in epileptic patients (22-24). This approach which offers an *in vivo* assessment of potential seizure-related down-regulation of GABA_A receptors has been successfully

combined with other diagnostic tools including 2 -deoxy- 2 -(18F)fluoro-d-glucose (FDG) PET, and electrocorticographic (ECoG) to provide a comprehensive evaluation of patients (20). However, expansion of these potentially diagnostic tools of refractory epilepsy requires a better understanding of molecular biomarkers of epilepsy and the expansion of the repertoire of radioactive ligands to reliably identify molecular dysfunctions associated with a risk for refractory epilepsy. In addition, advances in pharmacogenomics and in our understanding of the time course of molecular changes associated with epileptogenesis may provide new avenues to elucidate the basic mechanisms of pharmacoresistance and to elaborate useful predictive tools. Many different experimental models have been developed in order to investigate the basic mechanisms of epilepsy and the challenges posed by medical refractoriness. In this review, we will briefly describe some of these models, with emphasis on MTLE which is a form of epilepsy that critically affects the limbic system. Furthermore, we will describe the major electrophysiological features of the epileptiform activity in structures of the limbic system as well as the molecular alterations in ion channels and receptors that may support the notion that MTLE is a complex form of acquired channelopathy.

3. MODELS OF CONVULSIONS *VERSUS* MODELS EXHIBITING RECURRENT SPONTANEOUS SEIZURES

For many years, acute seizure models have played an essential role in epilepsy research including the screening and development of new antiepileptic drugs. Studies in acute animal models allowed the discovery of the PDS as a cellular correlate of surface epileptiform activity (*i.e.*, interictal spikes) (10). Traditional acute models induced in otherwise normal animals have been instrumental in early investigations related to the cellular basis of ictal discharge and termination. In these models, seizures are induced by either an electrical stimulation or a convulsant agent in non-epileptic “normal” mice, *i.e.* the maximal electroshock seizure (MES) (25-27), the maximal electroshock seizure threshold (MEST) test (28), the subcutaneous pentylenetetrazol seizure test (29), and the intravenous pentylenetetrazol seizure test (30-32). These tests can be used to characterize anticonvulsant and/or proconvulsant properties of compounds in mice in drug-screening programs (29,33-38). Although the parameters of these tests are optimized for mice, similar tests, with some modifications, can be used with rats. Interestingly, the vast majority of these acute models does not induce convulsive activity in the limbic system. Although these models are still used as a fast track screening for antiepileptic drugs, acute convulsions are induced in a “normal” brain which do not express the pathogenic features of chronic epilepsy. Compelling evidence indicates that molecular targets for antiepileptic drugs can be altered during the process of epileptogenesis (12,199-204,230-237). In addition, networks are remodeled due to seizure-mediated neuronal death and synaptic reorganizations. Furthermore, the seizures in these models can be used for testing of antiepileptic drug effects. A comparison of the

pharmacology of chronic models with models of acute (reactive or provoked) seizures in previously healthy (non-epileptic) animals, such as MES, demonstrates that drug testing in chronic models of epilepsy yields data which are more predictive of clinical efficacy and adverse effects, so that chronic models should be used relatively early in drug development to minimize false positives (39). Accordingly, new rational paradigms adopted by the pharmacological industry include pre-clinical testing of potentially antiepileptic and disease-modifying compounds in chronic animal models of epilepsy. Interestingly, the models that are now considered the gold standard for the pharmacological industry are animal models of MTLE in which epileptiform activity originates in the limbic system (40-42).

The advantage of chronic models in the development of novel antiepileptic drugs has been emphasized after the discovery of levetiracetam (namely Keppra®), a new wide spectrum antiepileptic drug which is highly efficient as monotherapy or adjunctive therapy of several epileptic syndromes (41,43-56). Levetiracetam is an antiepileptic drug devoid of anticonvulsant activity in the MES and pentylenetetrazole seizure tests, in both mice and rats. This contrasts a potent seizure suppression in genetic and kindled mice and rats and against chemoconvulsants inducing partial seizures in rats (57). The highly selective action in “epileptic” animals distinguishes levetiracetam from classic and other new AEDs that have nearly equipotent effects in normal and “epileptic” animals (58).

3.1. Chronic epilepsy models with limbic epileptogenesis

Human MTLE is characterized by maladaptive changes in neuronal networks including neuroanatomical changes (*i.e.*, neuronal loss, synaptic reorganization) and molecular abnormalities (*i.e.* abnormal expression of ion channels, receptors and transporters). Therefore, seizure-related rearrangements of the neuronal circuits and dynamic changes underlying epileptogenesis in human epilepsy is definitely better resembled by chronic models of MTLE. Accordingly, several animal models have been developed to investigate basic mechanisms underlying epileptogenesis. There are two popular MTLE models, which are known and characterized by the method of MTLE induction: (a) focal electrical stimulation and (b) systemic or intrahippocampal administration of neurotoxins (*i.e.*, kainic acid and pilocarpine) leading, in most cases, to *status epilepticus*. In addition, a rare genetic model of MTLE (Ihara's epileptic rat) has been developed by successive mating and selection from an inherited cataract rat (59). This model exhibits genetically programmed neuronal microdysgenesis in the hippocampus which has been causally related to the recurrent spontaneous seizures that affected animals develop (59,60).

3.2. Electrically-induced animal models to investigate MTLE

A number of variants of electrically-induced models have been described using different stimulation protocols and brain areas. These models can be classified according to whether electrical stimulation protocols induce (a) kindling or (b) electrically-induced self-sustained

"limbic" *status epilepticus* (SSLSE). In general, these models have been developed by stimulation of limbic structure (*i.e.*, hippocampus, amygdala). The kindling model was originally proposed by Goddard and colleagues in 1960 after stimulation of the hypothalamus and amygdala (61,62), who broadly defined it as "a relatively permanent alteration in brain function which results from repeated electrical or chemical stimulation and culminates in the appearance of electrographic and behavioral convulsions whenever the original stimulus is reapplied" (61). The word *kindling* is a metaphor: the increase in response to small stimuli is similar to the way small burning twigs can produce a large fire (61,63-65). This concept applies not only to electrically- but also to chemically-induced decreases in threshold for afterdischarge and seizures, and more broadly as circuit modifications and reduced threshold for hyperexcitability triggered by endogenously-generated abnormal electrical activity (*i.e.* seizures). In fact, the assertion that "seizure begets seizures" fits very well with this epileptogenesis process, implying long-lasting and dynamic reorganization of neuronal circuits in epileptiform activity (61,66-68). Mechanisms related to the induction of kindling, were also proposed as a pathological extreme in a continuous array of mechanisms (plasticity-pathology continuum model) (69) responsible for neuronal plasticity (*i.e.*, long-term potentiation of synaptic strengths) (69-73). Although it has been reported that repeated seizure stimulation can result in neuroplastic rearrangements of the limbic circuits (limbic epileptogenesis), spontaneous seizures are rare and not universal across laboratories or species. Therefore, there is currently no consensus in the literature whether kindling causes spontaneous seizures or not (39,74-81). Moreover, it is a matter of significant debate whether experimental results obtained in kindled animals apply to humans with epilepsy (74,75). (74) Although kindling allows the researcher to control and induce epileptiform activity upon stimulation, the low incidence of spontaneous recurrent seizures has prompted scientists to question whether this model produces a true epileptic state (75). It has been proposed that the neuronal alterations produced by kindling, including cell loss and aberrant axonal sprouting, are relatively mild and may not be sufficient to mediate epileptogenesis (82-85). Moreover, it is also not established whether neuronal damage and circuit reorganization observed in kindling is the result of, and not the cause of seizures.

3.3. Self-sustained limbic *status epilepticus* models

In addition to kindling, continuous electrical stimulation of limbic structures including hippocampus (86-90) and amygdala (91-93) has been used to trigger self-sustained limbic *status epilepticus* (SSLSE). Hippocampal stimulation-induced SSLSE is characterized by intense, but intermittent motor seizure activity, like that seen with kindled motor seizures and nearly continuous 'limbic' behavioral seizures, identical to those seen after low doses of kainic acid or during the early stages of kindling (90). The presence of synchronous, stimulus-independent seizure activity bilaterally in the hippocampi during stimulation precedes the establishment of SSLSE after hippocampal stimulation. Once established, SSLSE can persist for

several hours and is followed by chronic neuropathological changes reminiscent of hippocampal sclerosis encountered in epileptic patients (89). It has previously been shown that prolonged (60-min) low-intensity electrical stimulation of a kindled focus in the basolateral nucleus of the amygdala of Wistar rats can result in the development of SSSE with predominantly partial seizures and subsequent brain damage in the ipsilateral hemisphere (91). SSLSE initiates a cascade of events that lead to the development of spontaneous seizures in stimulated animals. Histologic examination of the amygdala and hippocampus from animals subjected to SSLSE revealed neuronal loss in the amygdala, hippocampus, and surrounding cortical areas, as well as mossy fiber sprouting in the dentate gyrus (89,93). *In vitro* studies using hippocampal slices taken from SSLSE epileptic have shown abnormal excitatory and inhibitory neurotransmission in CA1 area (86-88). Using extracellular and intracellular recording techniques in brain slice preparations from post-SSLSE model of chronic MTLE (1-7 months following SSLSE) these authors described hyperresponsiveness of the CA1 and dentate gyrus areas when compared to control tissue. "Epileptic" hyperresponsiveness was characterized by 2 or more population spikes elicited by a stimulus strength giving rise to maximal-amplitude population spikes. Control tissue never exhibited > 2 population spikes in either CA1 or dentate gyrus in response to similar stimuli (94). Moreover, monosynaptic excitatory postsynaptic potentials (EPSPs) evoked in CA1 pyramidal cells in post-SSLSE tissue were always longer than those evoked in control tissue, irrespective of whether hyperresponsiveness was present or not. EPSPs elicited by stimulus subthreshold for action potentials in post-SSLSE and in control slices and matched in amplitude had a statistically longer duration in the post-SSLSE slices (88).

Induction of *status epilepticus* (SE) is considered a reliable strategy to trigger persistent abnormalities (epileptogenesis) resulting in a chronic epileptic condition. Although electrically-induced SSLSE has been adopted in several epilepsy research laboratories, the most popular models are the kainic acid and the pilocarpine model of MTLE which are based on the induction of SE and subsequent epileptogenic changes leading to spontaneous recurrent seizures. There are different variants of these models according to the route and area of administration, doses, and combination with other excitotoxins. For instance, kainic acid, a powerful neurotoxic analogue of glutamate (95,96), has been administered systemically (97), intraventricularly (98) or intracerebrally (*e.g.*, intrahippocampal and intra-amygdaloid microinjection) (99-103) to induce focal and secondarily generalized SE and persistent limbic neuropathology, limbic hyperexcitability and recurrent spontaneous seizures (104,105). Accordingly, the kainic acid model is considered an important tool to investigate MTLE (106). Surface and depth electroencephalogram (EEG) recordings following intravenous injections of 1 mg/kg kainic acid reveal the presence of high frequency oscillations and spikes in the hippocampus, without propagation to other structures (107). When injected at doses above 4 mg/kg, kainic acid induces electrical seizures in limbic structures, similar to

those seen in MTLE. Kainic acid (0.1 to 1.0 μM) also induces spontaneous epileptiform spikes, originating in CA3, and increased evoked potentials in hippocampal slices *in vitro* (108). Moreover, hippocampal slices prepared from kainic acid-treated chronic epileptic rats reveal signs of hyperexcitability (*i.e.*, hyper-responsiveness, evoked and spontaneous epileptiform activity) in limbic structures. For instance, about 55% of the CA1 pyramidal cells, in slices where the CA3/CA4 region has been lesioned using intracerebroventricular kainic acid injection, respond with bursts of action potentials (synchronous hyperexcitability) after orthodromic excitation, which was associated with a loss of synaptic inhibition (109). Supporting the defective inhibition hypothesis, Nakajima *et al.*, (1991) reported weakened interconnections between pyramidal cells and interneurons in hippocampal slices dissected 2-4 weeks following bilateral intraventricular KA injections (110). Hippocampal slices from animals that had previously experienced continuous hippocampal stimulation-induced SSLSE demonstrated an increased excitability relative to slices from control animals as evidenced by epileptiform bursting in increased extracellular potassium ($(\text{K}^+)_0$) and decreased extracellular calcium ($(\text{Ca}^{2+})_0$) (89). These electrophysiological changes are associated with marked neuronal loss and reorganization of synaptic circuits in the hippocampus (96,108,111-113). The mechanisms underlying kainic acid-induced *status epilepticus* and subsequent epileptogenesis are considered to be mediated by selective activation of kainate receptors augmenting excitatory glutamatergic synapses in critical epileptogenic areas, such as the CA3 region of the hippocampus (114-117).

In the last decades, the pilocarpine model has become the most popular and widely used rodent model of MTLE (118). This model has been developed by the administration of high doses of the cholinergic agonist pilocarpine (320-400 mg/kg) to rats and mice. Pilocarpine administration, at doses above 400 mg/kg, resulted in a greater likelihood of induction of the complete syndrome and reduced latency to SE, but also increased mortality rate (119,120). In addition, the pilocarpine model has been also proposed as a rodent model to mimic chemical warfare nerve agent exposure (121). A variant of the pilocarpine model consists in the induction of SE by the combination of lithium pre-treatment (LiCl, *i.e.*, 3 mEq/kg) followed 20 hours later by a small dose of pilocarpine (25-30 mg/kg) (119,122-131) in adult rats. Subcutaneous administration of pilocarpine to rats that were pretreated with a small dose of lithium chloride results in the evolution of generalized convulsive *status epilepticus* (132). Interestingly, in contrast to rats, pretreatment with lithium does not potentiate the convulsant effect of pilocarpine in mice (133). Pre-treatment with lithium allows a significant reduction of the pilocarpine dose required to induce *status epilepticus* and results in a higher percentage of animals developing *status epilepticus*. Furthermore, a new variant of the model has been recently developed by intrahippocampal injection of pilocarpine (134,135). In most of these variants of the pilocarpine model, a blood-brain barrier non-permeable derivative of scopolamine (*e.g.*,

methyl-scopolamine, 1 mg/kg) is administered 30 minutes prior to pilocarpine, to minimize systemic cholinergic side effects including excessive salivation, diarrhea, etc). The mechanisms by which pilocarpine induce *status epilepticus* are not completely clear, but it is thought that overstimulation of muscarinic receptors by pilocarpine triggering hippocampal hyper-synchronization and secondary activation of glutamatergic receptors lead to *status epilepticus* (136,137). In the first minutes following pilocarpine injection, the animals experience head nodding, wet dog shakes, brief rearing consistent with activation of limbic pathways. These symptoms are followed by motor seizures (tonic clonic movements) lasting several seconds (usually less than 30 sec). After 2-3 of these seizures, animals enter in *status epilepticus* that can last several hours unless pharmacological intervention is performed. In most laboratories, convulsive behavior during *status epilepticus* is mitigated by diazepam (*i.e.*, 10-20 mg/kg) administered 2-4 hours after *status epilepticus* onset. This approach has been considered to improve mortality rate of the procedures (138-141). The duration of *status epilepticus* is critical for the development of the chronic phase. According to Lemos and Cavalheiro (1995), rats undergoing 30-min long SE and treated with a single (*i.p.*) injection of diazepam (10 mg/kg) and pentobarbital (30 mg/kg) do not develop spontaneous seizures (142). In contrast, animals presenting with SE lasting 1, 2, 6 or several hours exhibit latent periods of 52, 38, 17 and 14 days, respectively. However, it has also been reported that the latent period may be progressively shortened by decreasing the status epilepticus duration (143). Moreover, a recent study based on continuous video-EEG recordings revealed that the first spontaneous seizures occur 7.2 ± 3.6 days after 2h of pilocarpine-induced *status epilepticus* (144). These data indicate that epileptogenesis toward a chronic epileptic state may develop in a relatively short "latent" period following status epilepticus. Duration of 3 hours of the *status epilepticus* has been used in several studies to induce a chronic model with a relatively high frequency of recurrent spontaneous seizures when compared to a full-bloom non-interrupted *status epilepticus* (138-141). An advantage of limiting the duration to 3-4 hours is that *status epilepticus*-induced insult and consequent long-term modifications are similar across different animals in the same study, while *status epilepticus* of different durations may result in a wide range of cytoarchitectural and molecular abnormalities and probably very different seizure frequencies. Hence, data obtained from these animals will exhibit a large variability that may pose critical challenges for statistical analysis.

3.3.1. *In vivo* epileptiform activity in hippocampus during pilocarpine-induced *status epilepticus*

Previous studies using electroencephalographic recordings have reported that pilocarpine can evoke both ictal and interictal epileptic events. Low voltage, fast activity first appears in neocortex and amygdala, while a clear pattern of theta rhythm is evident in the hippocampus. When the behavioral manifestations become more severe, high voltage, fast EEG activity replaces the hippocampal theta rhythm (118,145-147). Moreover, at later stages, animals develop electrographic seizures, characterized by

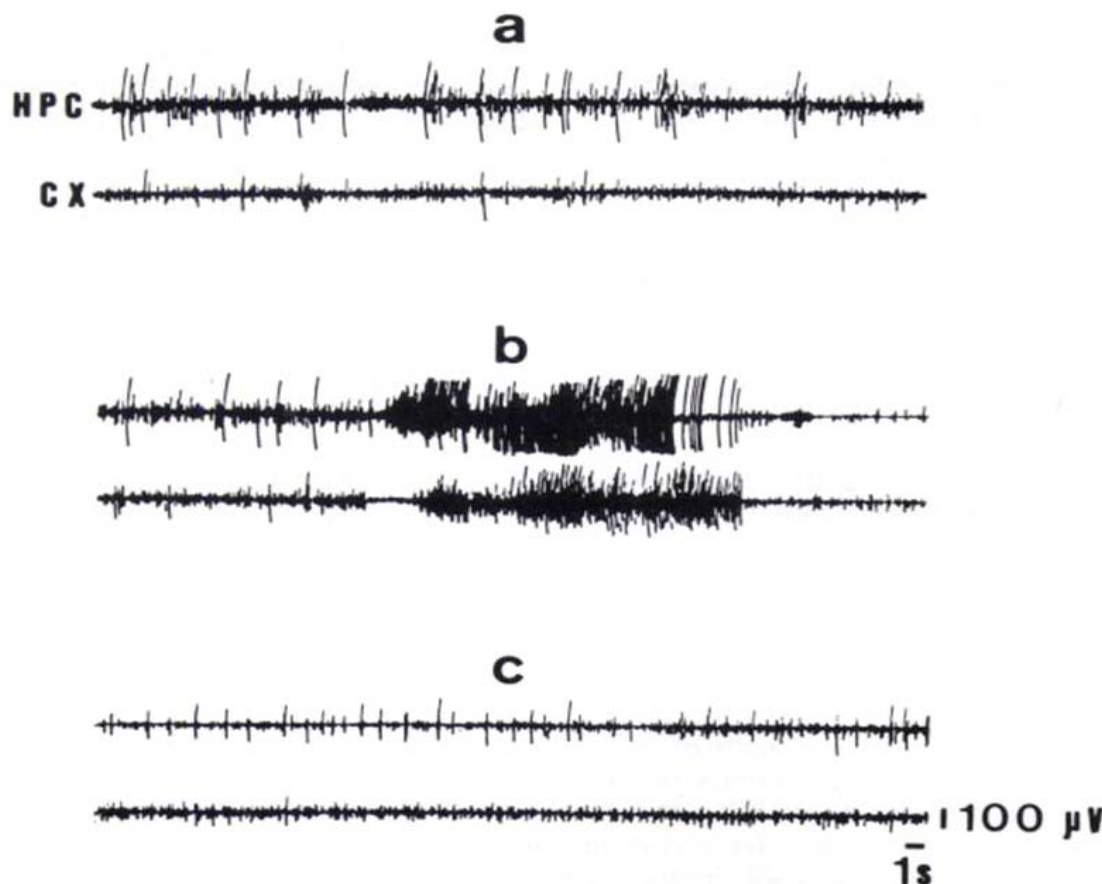


Figure 1. Electrographic recordings illustrating the buildup of a spontaneous recurrent seizure activity in a pilocarpine-treated chronically epileptic rat. The basal activity in the hippocampus (HPC) and cortex (CX) with isolated spikes is replaced by a burst of high voltage beginning in the HPC and spreading to CX. A, b, and c are continuous recordings. Figure adapted from Cavalheiro, 1995 (137).

high voltage, fast activity and prominent high voltage spiking that precedes seizures, most likely due to muscarinic system activation (118,145).

3.3.2. *In vivo* epileptiform activity during pilocarpine-induced spontaneous recurrent seizures

EEG recordings performed during the chronic phase of the model revealed that spontaneous seizures in pilocarpine-treated animals are characterized by bursts of spiking activity in the hippocampus that spread to the neocortex in 90% of the cases (137) (Figure 1). According to that study, electrographic seizures rarely last more than 60 s and are followed by depressed background activity with frequent EEG interictal spikes. Bursts of spiking activity are not observed in the neocortex alone (148). In addition, interictal activity is more intense when animals are seizure-free and during the sleep period while they are almost undetectable during motor activity and paradoxical sleep (149).

3.3.3. *In vitro* epileptiform activity in slices obtained from pilocarpine-treated epileptic animals

Different modalities of electrophysiological recordings have demonstrated the presence of abnormal

epileptiform activity in the limbic system of animal models of MTLE. *In vitro* electrophysiological studies on neurons and neuronal pathways of the limbic system have been developed using extracellular, intracellular and patch-clamp recordings in brain slices (hippocampal or entorhinal-hippocampal preparations). These studies have demonstrated the presence of epileptiform electrographic abnormalities in almost all the areas of the limbic system. For instance, evoked and spontaneous epileptiform electrical activity has been reported in CA3, CA1, subiculum and dentate gyrus areas of the hippocampus.

In vitro hyperexcitability and epileptiform activity have been described in brain slices from different brain areas of chronically epileptic rats. The major feature of the epileptiform activity is a PDS of the membrane potential as recorded by intracellular sharp microelectrodes or whole-cell patch-clamp (current clamp in bridge-mode) configuration. Bursts of action potentials are associated with a network burst, which is the emergence poly-spiking activity in extracellular recordings from neighboring areas. The normal responses obtained by extracellular recordings (field potentials) with the electrode positioned in the *stratum radiatum* of CA3 area upon *stratum lucidum*

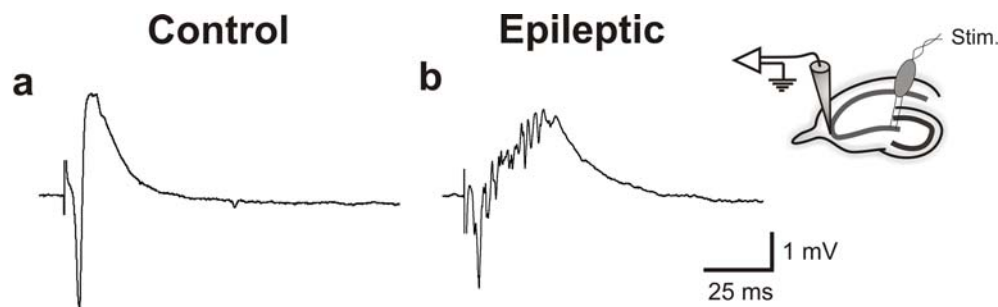


Figure 2. Hyper-responsiveness of hippocampal networks in hippocampal slices from pilocarpine-treated epileptic rats. A. In slices from saline-injected control rats, stimulation of mossy fiber pathway (*i.e.* bulk stimulation of hilar region) by a bipolar stimulator (*i.e.* 25 V) elicits a single population spike (field potential) in the CA3 area recorded with a glass microelectrode pipette (extracellular recordings) filled with 1 mM NaCl and positioned in the external border of the pyramidal layer. B. In contrast, similar paradigm evoked a “burst” of several population spikes (poly-spiking “epileptiform” activity) in the same region of hippocampal slices obtained from pilocarpine-treated chronic epileptic rats sacrificed at 40 days following status epilepticus.

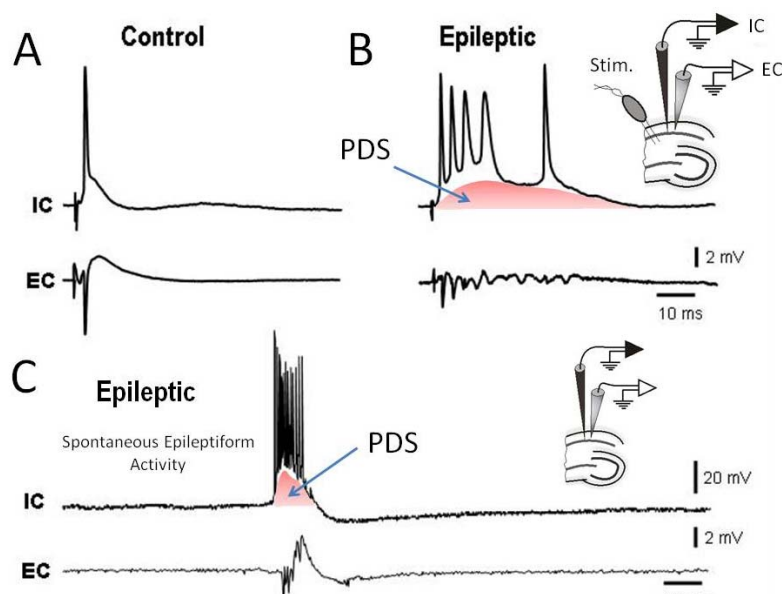


Figure 3. Epileptiform activity in the CA1 area of pilocarpine-treated epileptic rats. A. Supra-threshold stimulation of Schaffer collateral pathway trigger a single population spike recorded with extracellular (EC) electrodes positioned in the *stratum pyramidale* of CA1 area and a single action potential detected by intracellular (IC) electrode in pyramidal cell. In these experiments, the CA3 area was removed during slicing procedures. B. Under these conditions, similar paradigm evoked epileptiform (poly-spiking) activity in isolated CA1 area in slices from chronic epileptic rat. Notice that a in the intracellular recording, a burst of action potential emerge from a depolarizing wave (PDS) represented by the shadowed area. C. In this same recording configuration, spontaneous network epileptiform activity was detected in both electrodes indicating local hyperexcitability of CA1 area in epilepsy. Notice burst of action potentials during the PDS.

stimulation is characterized by a single population spike (Figure 2A). In contrast, a burst of multiple spikes (poly-spiking activity) can be generated in the same area of hippocampal slices obtained from pilocarpine-treated epileptic rats (Figure 2B). In addition to this hyper-responsiveness, the amplitude of the responses evoked in the epileptic tissue is usually smaller when compared to control tissue (138,150). It has been suggested that reduced amplitude results from extensive seizure-induced neuronal cell death in this area as a consequence of both the early epileptogenic insult (*status epilepticus*) and repetitive spontaneous seizures during the chronic phase of the

model. Other studies have reported hyper-responsiveness of the area CA1 both *in vivo* and *in vitro*. For instance, spontaneous and evoked epileptiform activities characterized by brief events of multiple low-amplitude population spikes have been recorded in the CA1 area in slices where the area CA3 has been removed (150). Epileptiform activity in this area is characterized by burst of action potentials arising from an underlying PDS recorded by intracellular sharp electrodes in association with poly-spiking activity as recorded by extracellular recordings in pyramidal area (Figure 3), but hyperexcitability foci may emerge at different locations in

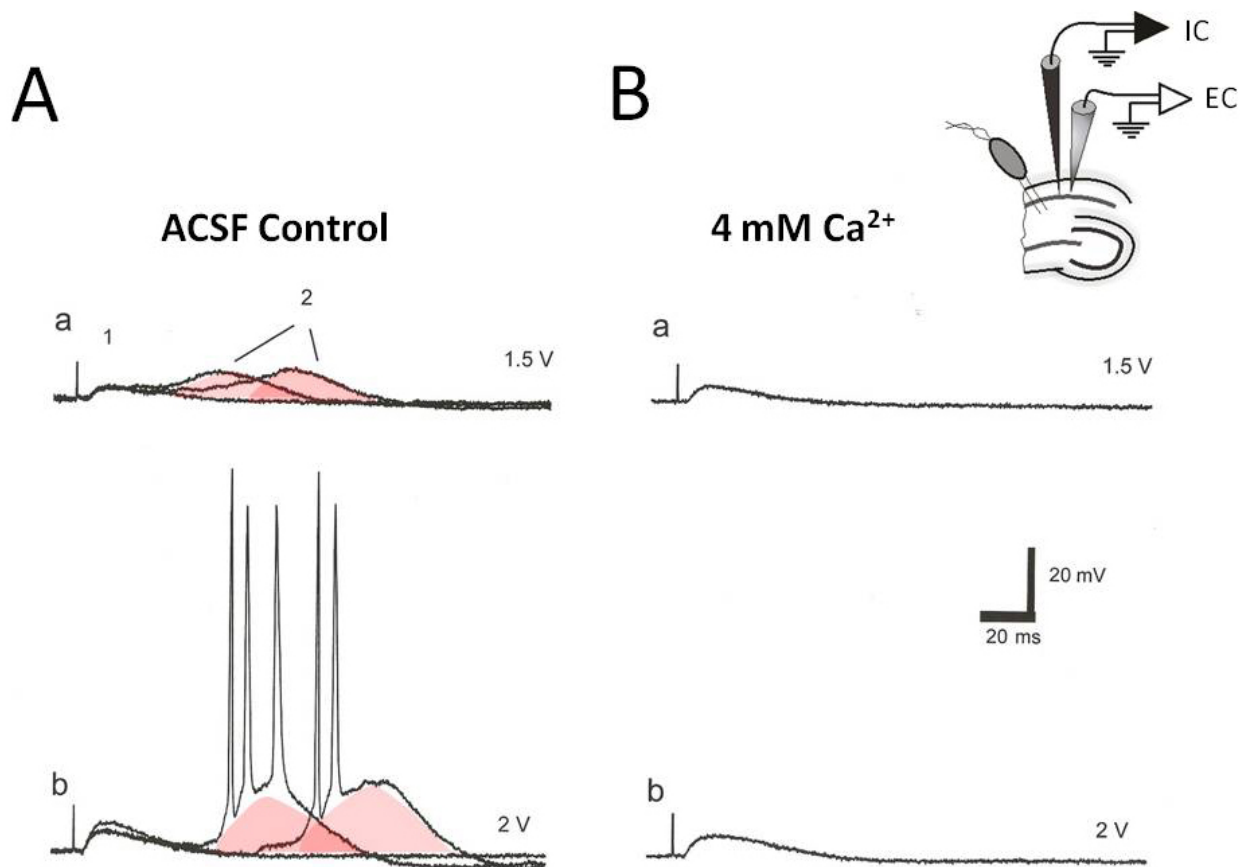


Figure 4. Abnormal excitatory connectivity in isolated CA1 area participates in the hyperexcitability observed during chronic epilepsy. A. A representative experiment in hippocampal CA1 micro-slice from chronic epileptic rat in standard ACSF (2 mM Ca^{2+}) revealing that Schaffer collateral stimulation trigger an initial excitatory postsynaptic potential (EPSP) (1) at low stimulation intensity (1.5V) that is followed by a “rebound” EPSPs (2) eventually eliciting burst of action potentials (“epileptiform activity”) riding depolarizing potentials (*i.e.* PDS) at higher stimulus intensity (2V) but still subthreshold for the first EPSP. B. Incubation of slices in ACSF containing higher concentration of divalent cations (4 mM Ca^{2+}) abolished the “rebound” epileptiform EPSPs and the burst of action potential at same stimulation intensities as in A.

the limbic system of chronic epileptic rats. Compelling evidence indicates that the genesis of epileptiform discharges in CA1 area, and probably other limbic zones are mediated by different mechanisms including abnormal intrinsic properties of neurons (*i.e.*, enhanced intrinsic bursting in the firing patterns, lack of accommodation), increased glutamatergic excitation due to abnormal NMDA and/or AMPA receptor function and expression, and reduced GABAergic inhibition (151-153). Sanabria *et al.*, (2001) have proposed that activity of spontaneous “bursting” neurons in CA1 can synchronize and recruit the local neurons into a network burst (interictal like activity). However, spontaneous epileptiform activity is blocked by small concentration (*i.e.*, 10 μM) of CNQX (150) or by an increase in divalent cation (*i.e.* Ca^{2+}) concentrations in the artificial cerebrospinal fluid (ACSF) (Figure 4) suggesting that recurrent excitatory connections play an important role in the recruitment of neurons into epileptiform activity (“network burst”). In general, these findings are consistent with abnormalities of intrinsic properties and dysfunction of network connections and neurotransmission (excitatory

and inhibitory) as has been classically proposed by the “epileptic neuron” and “epileptic aggregate” hypothesis to explain genesis of epileptic activity (154-157).

One of the distinctive features of the electrophysiology in MTLE is that responses evoked in the dentate gyrus following perforant path stimulation are, in most cases, not epileptiform. Cumulative evidence indicates that dentate gyrus functions as a filter that limits the propagation of epileptiform activity to limbic structures. Nonetheless, it is considered that during MTLE, this filter function is transiently altered prior to seizures. The mechanisms proposed for the transient disruption of dentate gyrus filter function are diverse. Dentate granule cells are characterized by low levels of excitability, an important aspect of hippocampal function, which distinguishes them from other principal cells of the hippocampus. For instance, recent studies indicate that, in contrast to pyramidal neurons, granule cells are intrinsically less excitable in the dentate gyrus of chronic epileptic rats and in human tissue removed during surgery for the treatment of drug-refractory

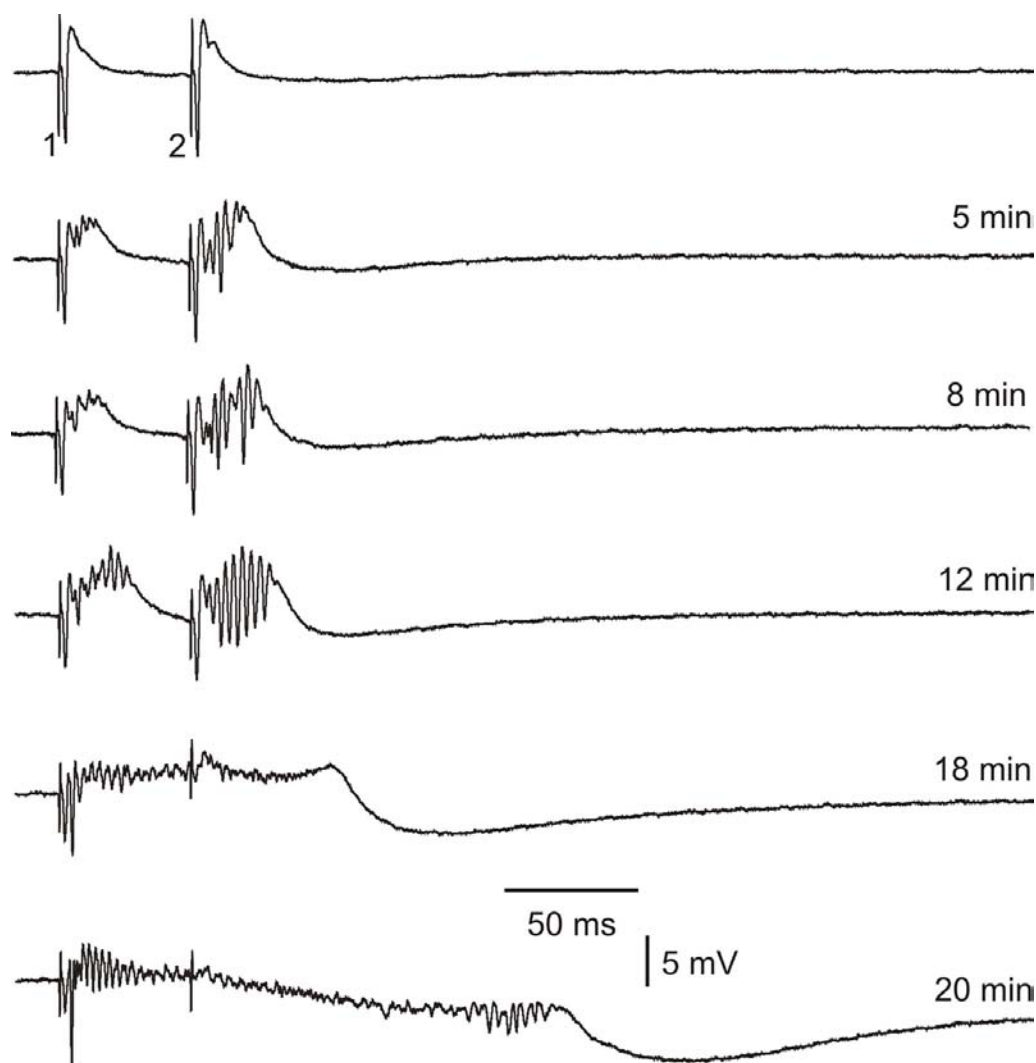


Figure 5. Epileptiform activity induced in hippocampal slice incubated in artificial cerebrospinal fluid containing 10 μ M of Bicuculline. Population spikes were recorded from the stratum pyramidale in CA3 area after paired stimulation of mossy fiber pathways (50 ms inter-stimulus interval, 1 and 2, stimulus artifacts) using electrode positioned in the hilus of dentate gyrus. Notice the genesis and progressive nature of epileptiform activity over time after application of bicuculline characterized by increase in the number of population spikes and prolongation of the network burst.

epilepsy (158,159). These findings play down the role of granule cell intrinsic excitability as one of the causes leading to disruption of dentate filter function. Moreover, it is widely accepted that network changes including transient disinhibition and excessive recurrent excitatory, mostly glutamatergic connectivity, play a pivotal role in the genesis and/or spreading of epileptiform activity through the dentate gyrus in MTLE (151). Supporting this notion, a large number of studies have reported an excess of glutamatergic recurrent excitation onto dendrites of granule cells as part of the active synaptic reorganization process associated with epileptogenesis (mossy fiber sprouting) (160-163). Interestingly, an increase in the GABAergic innervations has been also described in this area, although a recent electron microscopy and electrophysiological study indicates that the majority of the new connections are excitatory (151,164-166).

3.3.4. *In vitro* models of acute epileptiform activity in limbic structures from normal animals

Several *in vitro* models have been developed to induce epileptiform activity in limbic areas from animals that are normal. Different types of convulsants (*i.e.*, 4-aminopyridine, bicuculline, pilocarpine, etc) can trigger electrical stimulus-evoked and spontaneous epileptiform activity in slices from hippocampus and other limbic structures (167-169) (170). The mechanisms of these pro-convulsive agents are quite different, for instance bicuculline blocks GABA_A receptors inducing a disinhibition as a powerful stimulus to evoke an increase in excitability characterized by an increase in the number of population spikes and interictal like events (Figure 5). Changes in the ion concentration have been used as a manipulation to enhance excitability and induce *in vitro*

Table 1. “*In vitro*” models of acute epileptiform activity in slices from control (no epileptic) rats

Drugs and convulsants	Concentrations	Structure	Subfield	Reference
Pilocarpine	10μM	Hippocampus and entorhinal cortex	CA1-CA3	(170)
4-aminopyridine	50μM 100μM	Hippocampal Entorhinal cortex hippocampus-entorhinal cortex	CA1-CA2- CA3	(167-169)
Penicillin	2mM	Hippocampal	CA1-CA3	(309)
Bicuculline	10μM 20μM 100μM	Hippocampal	CA2-CA3	(168)
Veratridine	0.3μM	Hippocampal	CA1	(310)
Pentylenetetrazole	???	Hippocampal	CA3	(311)
Ions				
Ca ²⁺	Low: less than or equal to 0.2 mM	Hippocampus	CA1	(312,313)
Mg ²⁺	⁺ free Low high	Primary hippocampal cultures Organotypic cultured Organotypic cultures Amygdala	Different areas	(176,314-317) (318)
K ⁺	3 to 10mM	Parahippocampal	CA1-CA3	(319,320)
Organophosphate anticholinesterase soman	100nM	Hippocampus		(321,322)
Electrical				
Tetanic-like stimulation	100 Hz. 2 s in duration, once every 10 min, 10 episodes	Piriform-cortex hippocampal- parahippocampal		(323)
Train of pulses	60 Hz, pulse duration 100 us	Hippocampal	CA3	(169,324)

epileptiform activity. Among these modifications, increasing the concentration of potassium can enhance neuronal excitability. Pro-epileptic action of potassium increase is thought to be mediated by different mechanisms including increasing the number of intrinsically bursting neurons (171,172), reducing the driving force of potassium, reduction of GABA_A-mediated inhibition (173), swelling of neurons and increase in nonsynaptic interactions (174). In addition, low calcium and low magnesium can induce seizure-like events in the hippocampus (175-177). Although the mechanisms underlying the low-calcium induction of epileptiform activity are unknown, it has been demonstrated that removal of magnesium enhances N-methyl-D-Aspartate (NMDA) receptor-mediated currents (178,179) triggering an increase in glutamatergic excitation. The free-magnesium model of *in vitro* seizure activity in hippocampus has been used to evaluate the action of different anticonvulsant agents (180). Electrical stimulation (tetanus-like paradigms) has been also utilized to evoke *in vitro* epileptiform activity in limbic areas (Table 1). Acute models of *in vitro* epileptiform activity, especially in hippocampal slices, have been useful for screening potentially anticonvulsant compounds and in studying the mechanisms and pharmacology of epileptic activity (181-187). However, their predictive value for discovering new therapeutic drugs for epilepsy is hindered by the fact that ion channels, receptors and other molecular mechanisms in normal tissue are different than the ones expressed in chronically epileptic tissue.

4. GENETIC VERSUS ACQUIRED CHANNELOPATHIES

Channelopathies are diseases caused by impaired function of ion channel subunits or proteins which regulate them (188,189). These diseases may be either congenital (often resulting from a mutation or mutations in the encoding genes) or acquired (often resulting from autoimmune attack on an ion channel) (188,189).

Ion channel mutations have been causally implicated in a number of distinct disorders (188,189). For instance, congenital hyperinsulinism is caused by a mutation which results in deficiency in the expression of the inward-rectifying potassium channel subunit K_{IR}6.2 (190). In addition, most forms of myasthenia gravis, a neuromuscular disease leading to fluctuating muscle weakness and fatigability is a kind of acquired channelopathy caused by the production of antibodies directed against nicotinic acetylcholine receptors at the neuromuscular junction (191). Moreover, every human epilepsy syndrome for which a gene mutation has been identified is associated with dysfunction in ion channel subunits (192), characterizing inherited epilepsy as a genetic channelopathy. For instance, a mutation in the gene for K_v7.2 channel subunit causes the type 1 benign neonatal epilepsy (193), whereas mutation in the gene responsible for the α_{1H} channel subunit (Ca_v3.2) causes type 1 childhood absence epilepsy (194). In addition, sodium channel mutations that are associated with increased Na_v activity (e.g. Na_v1.1 channel subunit) can lead to enhanced seizure susceptibility, as in the severe myoclonic epilepsy of infancy GEFS+ type 2 (195-198).

In addition, ion channelopathy has been similarly hypothesized to play a role in acquired epilepsy due to central nervous system insult, such as *status epilepticus*. In this context, MTLE has been considered as a complex form of acquired channelopathy (12,199-204). While one may promptly figure out altered gene expression as a cause for acquired channelopathies, alternative splicing, altered protein processing, trafficking and recycling and post-translational modifications, such as protein phosphorylation may also contribute to an acquired ion channel dysfunction.

5. MTLE AS AN ACQUIRED CHANNELOPATHY

At a microscopy level, seizures and *status epilepticus* are known to produce multiple morphological abnormalities in the limbic system (i.e. loss of principal

Epileptiform activity in the limbic system

Table 2. Alterations in mRNA and protein levels of voltage-gated channels in MTLE

Channel family	Receptor (Common name/IUPHAR name/Gene name)	Model	Site	mRNA	Protein	Reference
Ca ²⁺ -Activated K ⁺ Channels	BK/KCa1.1/Kcnma1	Pilocarpine	Rat DG	↓	NA	(139)
Ca ²⁺ -Activated K ⁺ Channels	BK/KCa1.1/Kcnma1	Pilocarpine	Rat CA3	↓	↓	(268)
Cyclic Nucleotide- Regulated Channels	HCN1/HCN1/HCN1	Pilocarpine	Rat CA1	NA	↓	(199)
Cyclic Nucleotide- Regulated Channels	HCN1/HCN1/HCN1	Pilocarpine	Rat CA1	↓	NA	(204)
Cyclic Nucleotide- Regulated Channels	HCN1/HCN1/HCN1	Pilocarpine	Rat DG	↑	↑	(294)
Cyclic Nucleotide- Regulated Channels	HCN1/HCN1/HCN1	Human temporal lobe epilepsy	Human DG	↑	↑	(294)
Cyclic Nucleotide- Regulated Channels	HCN1/HCN1/HCN1	Kainate	Rat CA1	NA	↓	(303)
Cyclic Nucleotide- Regulated Channels	HCN1/HCN1/HCN1	Kainate	Rat CA1	↓	NA	(295)
Cyclic Nucleotide- Regulated Channels	HCN1/HCN1/HCN1	Kainate	Rat DG	↔	NA	(295)
Cyclic Nucleotide- Regulated Channels	HCN1/HCN1/HCN1	Amygdala kindling	Rat CA1	↔	NA	(295)
Cyclic Nucleotide- Regulated Channels	HCN1/HCN1/HCN1	Amygdala kindling	Rat DG	↔	NA	(295)
Cyclic Nucleotide- Regulated Channels	HCN2/HCN2/HCN2	Pilocarpine	Rat CA1	NA	↔	(199)
Cyclic Nucleotide- Regulated Channels	HCN2/HCN2/HCN2	Pilocarpine	Rat CA1	↓	NA	(204)
Cyclic Nucleotide- Regulated Channels	HCN2/HCN2/HCN2	Pilocarpine	Rat DG	↔	↔	(294)
Cyclic Nucleotide- Regulated Channels	HCN2/HCN2/HCN2	Human temporal lobe epilepsy	Human DG	↔	↔	(294)
Cyclic Nucleotide- Regulated Channels	HCN2/HCN2/HCN2	Kainate	Rat CA1	NA	↓	(303)
Cyclic Nucleotide- Regulated Channels	HCN2/HCN2/HCN2	Kainate	Rat CA1	↓	NA	(295)
Cyclic Nucleotide- Regulated Channels	HCN2/HCN2/HCN2	Kainate	Rat DG	↓	NA	(295)
Cyclic Nucleotide- Regulated Channels	HCN2/HCN2/HCN2	Amygdala kindling	Rat CA1	↔	NA	(295)
Cyclic Nucleotide- Regulated Channels	HCN2/HCN2/HCN2	Amygdala kindling	Rat DG	↔	NA	(295)
Inwardly Rectifying K ⁺ Channels	GIRK2/Kir3.2/KCNJ6	Kainate	Rat DG	↑	↑	(274)
Inwardly Rectifying K ⁺ Channels	GIRK2/Kir3.2/KCNJ6	Kainate	Rat CA1, CA3	↔	↔	(274)
Inwardly Rectifying K ⁺ Channels	IRK1/Kir2.1/KCNJ2	Kainate	Mouse DG	NA	↑	(158)
Inwardly Rectifying K ⁺ Channels	IRK2/Kir2.2/KCNJ12	Kainate	Mouse DG	NA	↑	(158)
Inwardly Rectifying K ⁺ Channels	IRK3/Kir2.3/KCNJ4	Kainate	Mouse DG	NA	↑	(158)
Inwardly Rectifying K ⁺ Channels	IRK3/Kir2.3/KCNJ4	Pilocarpine	Rat hippocampus	↓	NA	(271)
Inwardly Rectifying K ⁺ Channels	IRK3/Kir2.3/KCNJ4	Human temporal lobe epilepsy	Human temporal cortex	↓	↓	(270)
Inwardly Rectifying K ⁺ Channels	IRK4/Kir2.4/KCNJ14	Kainate	Mouse DG	NA	↑	(158)
Two-pore Weak Inwardly Rectifying K ⁺ Channels	Twik1/K2P1.1/KCNK1	Kainate	Mouse DG	NA	↑	(158)
Two-pore Weak Inwardly Rectifying K ⁺ Channels	Twik2/K2P6.1/KCNK6	Kainate	Mouse DG	NA	↑	(158)
Two-pore Weak Inwardly Rectifying K ⁺ Channels	TASK-1/K2P3.1/KCNK3	Pilocarpine	Rat DG	NA	↓	(272)
Two-pore Weak Inwardly Rectifying K ⁺ Channels	TASK-1/K2P3.1/KCNK3	Pilocarpine	Rat CA1	NA	↔	(272)
Two-pore Weak Inwardly Rectifying K ⁺ Channels	TASK-2/K2P5.1/KCNK5	Pilocarpine	Rat DG	NA	↑	(273)
Two-pore Weak	TASK-2/K2P5.1/KCNK5	Pilocarpine	Rat CA1	NA	↓	(273)

Epileptiform activity in the limbic system

Inwardly Rectifying K ⁺ Channels							
Two-pore Weak Inwardly Rectifying K ⁺ Channels	TASK-2/K2P5.1/KCNK5	Pilocarpine	Rat CA3	NA	↑		(273)
Voltage-Gated Ca ²⁺ Channels	T-type (α1G)/CaV3.1/CACNA1G	Pilocarpine	Rat CA1	↔	↔		(289)
Voltage-Gated Ca ²⁺ Channels	T-type (α1H)/CaV3.2/CACNA1H	Pilocarpine	Rat CA1	↑	↑		(289)
Voltage-Gated Ca ²⁺ Channels	T-type (α1I)/CaV3.3/CACNA1I	Pilocarpine	Rat CA1	↔	↔		(289)
Voltage-Gated Ca ²⁺ Channels	L-type (α1C)/CaV1.2/CACNA1C	Pilocarpine	Rat hippocampus	↑	↑		(290)
Voltage-Gated Ca ²⁺ Channels	L-type (α1C)/CaV1.2/CACNA1C	Human temporal lobe epilepsy	Human DG	NA	↓		(292)
Voltage-Gated Ca ²⁺ Channels	L-type (α1D)/CaV1.3/CACNA1D	Human temporal lobe epilepsy	Human DG	NA	↑		(292)
Voltage-Gated Ca ²⁺ Channels	L-type (α1D)/CaV1.3/CACNA1D	Pilocarpine	Rat hippocampus	↑	↑		(290)
Voltage-Gated Ca ²⁺ Channels	P/Q-type (α1A)/CaV2.1/CACNA1A	Pilocarpine	Rat hippocampus	↑	↑		(290)
Voltage-Gated Ca ²⁺ Channels	P/Q-type (α1A)/CaV2.1/CACNA1A	Kainate	Rat DG	↑	NA		(291)
Voltage-Gated Ca ²⁺ Channels	P/Q-type (α1A)/CaV2.1/CACNA1A	Human temporal lobe epilepsy	Human DG, CA3	NA	↑		(292)
Voltage-Gated Ca ²⁺ Channels	N-type (α1B)/CaV2.2/CACNA1B	Human temporal lobe epilepsy	Human DG	NA	↑		(292)
Voltage-Gated Ca ²⁺ Channels	R-type (α1E)/CaV2.2/CACNA1B	Human temporal lobe epilepsy	Human DG	NA	↑		(292)
Voltage-Gated Ca ²⁺ Channels	β1/CACNB1	Human temporal lobe epilepsy	Human CA	NA	↑		(293)
Voltage-Gated Ca ²⁺ Channels	β2/CACNB2	Human temporal lobe epilepsy	Human CA	NA	↑		(293)
Voltage-Gated Ca ²⁺ Channels	β3/CACNB3	Human temporal lobe epilepsy	Human CA	NA	↔		(293)
Voltage-Gated Ca ²⁺ Channels	β4/CACNB4	Human temporal lobe epilepsy	Human CA	NA	↔		(293)
Voltage-Gated Na ⁺ Channels	NaV1.1/SCN1A	Human temporal lobe epilepsy	Human hippocampus	See legend	NA		(325)
Voltage-Gated Na ⁺ Channels	NaV1.1/SCN1A	Human temporal lobe epilepsy	Human hippocampus	↔	NA		(278)
Voltage-Gated Na ⁺ Channels	NaV1.1/SCN1A	Pilocarpine	Rat DG	↔	NA		(200)
Voltage-Gated Na ⁺ Channels	NaV1.1/SCN1A	Pilocarpine	Rat CA1	↔	↔		(279)
Voltage-Gated Na ⁺ Channels	NaV1.2/SCN2A	Human temporal lobe epilepsy	Human hippocampus	See legend	NA		(325)
Voltage-Gated Na ⁺ Channels	NaV1.2/SCN2A	Human temporal lobe epilepsy	Human hippocampus	↓	NA		(278)
Voltage-Gated Na ⁺ Channels	NaV1.2/SCN2A	Pilocarpine	Rat DG	↓	NA		(200)
Voltage-Gated Na ⁺ Channels	NaV1.3/SCN3A	Human temporal lobe epilepsy	Human hippocampus	↑	NA		(278)
Voltage-Gated Na ⁺ Channels	NaV1.3/SCN3A	Pilocarpine	Rat DG	↔	NA		(200)
Voltage-Gated Na ⁺ Channels	NaV1.4/SCN4A	Human temporal lobe epilepsy	Human hippocampus	↔	NA		(278)
Voltage-Gated Na ⁺ Channels	NaV1.5/SCN5A	Pilocarpine	Rat DG	↔	NA		(200)
Voltage-Gated Na ⁺ Channels	NaV1.6/SCN8A	Pilocarpine	Rat DG	↓	NA		(200)
Voltage-Gated Na ⁺ Channels	NaV1.6/SCN8A	Pilocarpine	Rat CA1	↑	↑		(279)
Voltage-Gated Na ⁺ Channels	NaVβ1/SCN1B	Human temporal lobe epilepsy	Human hippocampus	NA	↑		(281)
Voltage-Gated Na ⁺ Channels	NaVβ1/SCN1B	Pilocarpine	Rat DG	↓	NA		(200)
Voltage-Gated Na ⁺ Channels	NaVβ2/SCN2B	Pilocarpine	Rat DG	↔	NA		(200)
Voltage-Gated Na ⁺ Channels	NaVβ3/SCN3B	Human temporal lobe epilepsy	Human hippocampus	NA	↓		(282)
Voltage-Gated Na ⁺ Channels	NaVβ3/SCN3B	Human temporal lobe epilepsy	Human hippocampus	↓	NA		(283)
Voltage-Gated K ⁺ Channels	Kv7.5/KCNQ5	Human temporal lobe epilepsy	Human CA1	NA	↓		(256)
Voltage-Gated K ⁺ Channels	Kv7.2/KCNQ2	Amygdala kindling	Rat amygdala	NA	↑		(326)

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Voltage-Gated Channels	K ⁺	Kv4.2/KCND2	Pilocarpine	Rat CA1	NA	↓	(251)
Voltage-Gated Channels	K ⁺	Kv4.2/KCND2	Pilocarpine	Rat DG	NA	↓	(251)
Voltage-Gated Channels	K ⁺	Kv4.2/KCND2	Pilocarpine	Rat CA1	↓	↓	(12)
Voltage-Gated Channels	K ⁺	Kv4.2/KCND2	Pilocarpine	Rat CA1, CA3	NA	↓	(252)
Voltage-Gated Channels	K ⁺	Kv4.3/KCND3	Pilocarpine	Rat DG	NA	↔	(251)
Voltage-Gated Channels	K ⁺	KChIP1/KCNIP1	Pilocarpine	Rat DG	NA	↓	(251)
Voltage-Gated Channels	K ⁺	KChIP1/KCNIP1	Pilocarpine	Rat CA1, CA3	NA	↓	(252)
Voltage-Gated Channels	K ⁺	KChIP2/ KCNIP2	Pilocarpine	Rat CA1	NA	↓	(251)
Voltage-Gated Channels	K ⁺	KChIP2/ KCNIP2	Pilocarpine	Rat DG	NA	↔	(251)
Voltage-Gated Channels	K ⁺	Kv1.4/KCNA4	Pilocarpine	Rat CA3	NA	↑	(251)
Voltage-Gated Channels	K ⁺	Kv1.4/KCNA4	Pilocarpine	Rat DG	NA	↑	(251)
Voltage-Gated Channels	K ⁺	Kv1.4/KCNA4	Pilocarpine	Rat DG	NA	↑	(251)

Alterations in mRNA and protein levels of voltage-gated channels in MTLE. ¹ Channel family according IUPHAR classification, ² Indicates the source of data (i.e. clinical or experimental model of MTLE), ³ Limbic area where experiments have been made. Abbreviations: ↑, increased levels; ↓, decreased levels; ↔, no change; NA, no data found.

cells, interneurons, gliosis, synaptic rearrangements, neurosynaptogenesis, neurogenesis (205-208) which may be manifested at different time frames after the epileptogenic insult (209-214). In fact, since the appearance of the first models of *status epilepticus* and epilepsy, numerous researchers have demonstrated immediate and/or long-lasting events that could modify brain excitability and result in the complex neurochemical alterations which contribute to seizure generation and spreading as well as pharmacoresistance in MTLE. These neurochemical alterations include disruption of ionic gradients across the plasma membrane, alterations in the synthesis, degradation, release and uptake of neurotransmitters, and changes in signaling pathways through the plasma membrane. More in-depth changes include synaptic remodeling and circuit changes, activation of inflammatory processes, reactive gliosis and programmed cell death (apoptosis) (215-229). However, key technological advances in the area of molecular biology during the last decade have been instrumental in deciphering the molecular aberrations underlying epileptogenesis. In this context, compelling data indicate that seizure-related modifications in expression and function of different ion channels and receptors can contribute to enhanced excitability and pharmacoresistance in epilepsy (12,199-204,230-237). Accordingly, since the development of global gene expression profiling techniques, an increasing number of publications has revealed differentially expressed genes in the process of epileptogenesis. Considering clinical and experimental studies on MTLE, approximately 2000 genes have been found to show differential expression during the latent and/or chronic phase of this condition (238,239). Given that the potential for complexity in genetic and molecular changes in MTLE is enormous, in this review we will focus in the changes in the expression and regulation of voltage-gated ion channels in MTLE, bringing together clinical and experimental evidence. Seizure-related changes in ion channels and receptors are area of special interest for the discovery of novel targets for antiepileptic and anti-

epileptogenic drugs (240,241). A summary of mRNA and protein levels of voltage-gated channels is shown in Table 2.

5.1. Potassium channels

Potassium (K⁺) channels are the largest and most diverse group of ion channels, represented by some 70 known loci in the mammalian genome (242). The voltage-gated K_V channels, in turn, form the largest family of some 40 genes among the group of human potassium channels, which also includes the Ca²⁺-activated (K_{Ca}), inward-rectifying (K_{IR}), and two-pore (K_{2P}) families (242-245).

Compelling data indicate that K⁺ channels critically modulate neuronal excitability and that activation of these channels crucially affect intrinsic membrane properties and action potential firing pattern of the neurons (e.g., firing frequency) (246,247). For instance, their activation limits the firing frequency of action potentials and is important for regulating afterhyperpolarization in central neurons and other types of electrically excitable cells (248). Accordingly, it has been acknowledged that depolarization-induced activation of K⁺ channels may provide a stabilizing influence by hyperpolarizing the membrane upon opening. Hence, blocking K⁺ channels and preventing them from opening in response to membrane depolarization decreases seizure threshold. Moreover, these channels are also expressed at presynaptic terminals, where they play a pivotal role in controlling neurotransmitter release (247-250). Therefore, deficit of K⁺ channels has been largely implicated in the pathogenesis of neurological disorders characterized by abnormal neuronal excitability, including epilepsy (188).

In light of the view of MTLE as an acquired channelopathy, the expression of K⁺ channels has been explored in different seizure and epilepsy models. Regarding the K_V family, mRNA and/or protein levels of A-type channels (i.e., somatodendritic K_V4.2, KChIP1 and

KChIP2) are decreased in the CA1 hippocampal subfield of pilocarpine-epileptic rats (12,251,252). Moreover, immunoreactivity for $K_v4.2$ was reduced in the hippocampus of seizure-sensitive gerbils when compared to seizure-resistance ones (253). Importantly, Bernard *et al.* (2004) have shown a significant increase in the percent of ERK phosphorylated- $K_v4.2$ in the hippocampal CA1 area of pilocarpine-epileptic rats. This finding indicates that post-translational modifications also contribute to hyperexcitability in MTLE, since phosphorylation of $K_v4.2$ channels by ERK shifts the activation curve toward more positive values, which decreases dendritic K^+ currents (254). In addition, a $K_v4.2$ channel gene mutation has been found in a MTLE patient, namely a $K_v4.2$ truncation mutation because the identified mutation resulted in a truncated $K_v4.2$ protein lacking the last 44 amino acids in the carboxyl-terminal (255). Interestingly, cells expressing such a $K_v4.2$ mutant channel showed attenuated K^+ current density, which is consistent with increased neuronal excitability in MTLE.

Levels of other types of K_v subunits have been also determined in experimental and clinical MTLE. For instance, protein levels of the $K_v7.5$ channels are reduced in the hippocampal CA1 subfield of patients with MTLE (256). In this context, it is worth mentioning that retigabine, a new drug in development for the treatment of epilepsy, activates subtypes of K_v7 channels responsible for M-current in neurons (232,257). Interestingly, retigabine possesses a broad spectrum anticonvulsive profile in animal seizure and epilepsy models (258-260). In contrast to the decrease in $K_v4.2$, KChIP1, KChIP2 and $K_v7.5$ channel subunits in pilocarpine-epileptic rats and in the epileptic human hippocampus, it has been shown that protein levels of $K_v1.4$ are increased in the rat CA3 hippocampal subfield and dentate gyrus (DG) in the pilocarpine model of MTLE (251).

The second major group of K^+ -selective channels consists of the K_{Ca} family (245). These channels are activated by rises in cytosolic calcium largely in response to calcium influx via voltage-gated calcium channels that open during action potentials (245). Therefore, K_{Ca} are fundamental regulators of neuronal excitability, and activation of these potassium channels controls a number of physiological processes, including firing properties of neurons (*i.e.*, interspike interval and spike-frequency adaptation) and control of transmitter release (261-265). Three broad families of calcium-activated potassium channels have been identified, which can be separated on both biophysical and pharmacological grounds (245). These have been termed BK (large conductance), SK (small conductance), and IK (intermediary conductance) channels (245). BK channels ($K_{Ca1.1}$) have recently been implicated in the pathogenesis of genetic epilepsy (266). In fact, a gain-of-function of BK channels may contribute to the epileptic phenotype (*i.e.*, temporal lobe seizures) (267), probably by inducing rapid repolarization of action potentials, which in turn contributes to generalized epilepsy and paroxysmal dyskinesia by allowing neurons to fire at a faster rate (266,267). On the other hand, the role of BK and other K_{Ca} in acquired MTLE has begun to be determined

only recently. In this context, quantitative analysis of mRNA by RT-PCR and protein levels by western blotting and immunohistochemistry of BK channels revealed that these channels are significantly reduced in the hippocampus of pilocarpine-epileptic rats when compared to age-matched non-epileptic control rats (139,268). In addition, a significant down-regulation of SK2 ($K_{Ca2.2}$) and SK3 ($K_{Ca2.3}$) but not SK1 ($K_{Ca2.1}$) channel mRNA and protein was detected in samples obtained from chronic pilocarpine-epileptic rats (269). Levels of members of the K_{IR} and K_{2P} families have been shown to be altered in MTLE. For instance, mRNA and protein levels of the IRK3 ($K_{IR2.3}$) channel are decreased in the temporal cortex of epileptic patients as well as in the hippocampus of pilocarpine-epileptic rats (270,271). In addition, in the pilocarpine model, expression of TASK-1 ($K_{2P3.1}$) and TASK-2 ($K_{2P5.1}$) are decreased in the DG and hippocampal CA1 subfield, respectively (272,273). On the other hand, it should be noted that increases in K^+ channels have been also found in some instances. For instance, mRNA and protein levels of the GIRK2 ($K_{IR3.2}$) channel are increased in the DG of kainate-epileptic rats (274), and TASK-2 ($K_{2P5.1}$) immunoreactivity is increased in the CA3 subfield and DG of pilocarpine-epileptic rats (273). In addition, in a study by Young *et al.* (2009), protein expression of K_{IR2} subunits ($K_{IR2.1}$, $K_{IR2.2}$, $K_{IR2.3}$, $K_{IR2.4}$) and of K_{2P} channels (Twik1/ $K_{2P1.1}$, Twik2/ $K_{2P6.1}$) was upregulated in granule cells taken from kainate-epileptic mice (158).

5.2. Voltage-gated sodium channels

Voltage-gated sodium channels (Na_v) are present in excitable cells, including nerve, muscle, and neuroendocrine cell types (275). Na_v are members of the superfamily of ion channels that includes K_v and voltage-gated Ca^{2+} channels; however, unlike K^+ and Ca^{2+} channels, the functional properties of known sodium channels are relatively similar (275). For instance, Na_v are responsible for action potential initiation with the fast, transient Na^+ currents (I_{NaF}) and enhance neuronal repetitive firing capacity with persistent Na^+ currents (I_{NaP}) (276,277). A variety of different sodium channels has been identified by electrophysiological recording, biochemical purification, and cloning. Na_v channels consist of a highly processed alpha subunit associated with auxiliary beta subunits (275). The pore-forming alpha subunit is sufficient for functional expression, but the kinetics and voltage dependence of channel gating are modified by the beta subunits (275).

It is generally believed that altered functioning of Na_v may be critical in conditions leading to the alteration of neuronal excitability, basically because of the pivotal role of Na_v in the modulation of neuronal firing properties. Moreover, recent genetic studies have implicated mutations in these channels in the pathogenesis of epilepsy (195-197), and these channels are common targets of antiepileptic drugs, such as phenytoin, carbamazepine and lamotrigine, possibly acting on the same binding site at clinically relevant concentrations (240,241). The expression of sodium channels has been investigated in experimental MTLE as well as in the human MTLE. For instance, the

mRNA levels for the pore-forming α subunit $\text{Na}_v1.2$ was persistently down-regulated up to 30 days following *status epilepticus* in the DG of pilocarpine-epileptic rats (200), as well as in the human epileptic hippocampus (278). In addition, mRNA levels for $\text{Na}_v1.6$ subunits are decreased in the rat DG in the pilocarpine model (200). On the other hand, $\text{Na}_v1.6$ mRNA and protein levels are increased in the rat hippocampal CA1 subfield in pilocarpine-epileptic rats (279), suggesting that regional differences in the alterations in the levels of Na_v subunits could exist. In addition, while $\text{Na}_v1.3$ mRNA levels are increased in patients with MTLE (278), no alterations in this Na_v subunit were detected in the rat DG in the pilocarpine model of epilepsy (200). Accordingly, several idiopathic epilepsy syndromes have been associated with mutations in *SCN1A* (the $\text{Na}_v1.1$ gene) (196-198), but no significant differences in the amount of $\text{Na}_v1.1$ mRNA or protein levels have been detected in the epileptic human or rat hippocampus (200,278,280).

Interestingly, reduced expression of the Na_v beta1 and beta2 auxiliary subunits has been associated with a depolarizing shift in Na_v inactivation, in such a way that the window current is amplified, resembling the increased I_{NaP} seen in animal models of MTLE (276,277). In this context, it has been shown that mRNA levels for the Na_v beta1 but not for the beta2 subunit are decreased in the DG of pilocarpine-epileptic rats, whereas protein levels for the beta1 subunit are increased in the hippocampus of MTLE patients (200,281). Moreover, two studies by van Gassen *et al.* (2007; 2009) showed a decrease in mRNA and protein levels of the beta3 subunit in the epileptic human hippocampus (282,283).

In addition to changes in the levels of Na_v subunits in MTLE, there is evidence for changes in the sensitivity of these channels to several antiepileptic drugs. In fact, the inhibitory effect of carbamazepine (CBZ) on Na^+ currents is decreased in DG and CA1 neurons taken from pilocarpine-epileptic rats (201,284). Moreover, the CBZ-elicited blockade of the experimentally induced epileptiform activity in the epileptic DG of human hippocampal slices is much greater in a CBZ-responsive than in CBZ-resistant patients (285,286). Altogether, these results indicate that the Na_v channels and possibly other molecular targets for antiepileptic drugs are structurally and/or functionally modified in MTLE, undergoing a form of plasticity which contributes to pharmacoresistance (42).

5.3. Voltage-gated calcium channels

Voltage-gated calcium channels (Ca_v) are members of a gene superfamily of transmembrane ion channel proteins that mediate calcium influx in response to membrane depolarization (287). Ca_v are complex proteins composed of four or five distinct subunits that are encoded by multiple genes. The α_1 subunit is the largest subunit, and it incorporates the conduction pore, the voltage sensor and gating apparatus, and most of the known sites of channel regulation by second messengers, drugs, and toxins (287). Most types of Ca_v are composed of auxiliary $\alpha_2\delta$, beta and gamma subunits, which modulate the properties of the channel complex, although the

pharmacological and electrophysiological properties of Ca_v arise primarily from the different α_1 subunits (287).

Calcium currents recorded in different cell types have diverse physiological and pharmacological properties (287,288). In neurons, the primarily expressed calcium currents are N-type, P/Q-type, and R-type, where they initiate neurotransmission at most fast synapses and mediate calcium entry into cell bodies and dendrites (287,288). T-type calcium currents are also expressed in neurons, and they are involved in shaping the action potential and controlling patterns of repetitive firing (287,288). In addition, L-type calcium currents, the main calcium currents recorded in muscle and endocrine cells, are also seen in neurons (287,288).

The expression levels Ca_v has been investigated in clinical and experimental MTLE. In a study by Becker *et al.* (2008), mRNA and protein levels of different T-type-forming α subunits were evaluated. The mRNA and protein levels for α_{1G} ($\text{Ca}_v3.1$) or α_{1I} ($\text{Ca}_v3.3$) were not altered in the hippocampal CA1 subfield of pilocarpine-epileptic mice, but levels of α_{1H} ($\text{Ca}_v3.2$) mRNA and protein were significantly upregulated (289). Interestingly, the appearance of spontaneous seizures was dramatically reduced in $\text{Ca}_v3.2$ knockout mice, suggesting transcriptional induction of this T-type calcium channel as a critical step in epileptogenesis in the pilocarpine model of epilepsy. In addition, it has been shown that immunostaining for the α_{1C} ($\text{Ca}_v1.2$) and α_{1D} ($\text{Ca}_v1.3$) subunits (which form L-type channels) and of the α_{1A} subunit (which form P/Q-type channels) are increased in the mice DG in the pilocarpine model of epilepsy (290). Moreover, increased mRNA levels of the α_{1A} ($\text{Ca}_v2.1$) subunit has been found in the DG of kainate-epileptic rats (291).

In temporal lobe epilepsy patients with Ammon's horn sclerosis, an increased immunoreactivity was observed for α_{1A} ($\text{Ca}_v2.1$), α_{1B} ($\text{Ca}_v2.2$), α_{1D} ($\text{Ca}_v1.3$) and α_{1E} ($\text{Ca}_v2.3$) in the DG, whereas immunoreactivity for the α_{1A} ($\text{Ca}_v2.1$) and α_{1C} ($\text{Ca}_v1.2$) were respectively increased and decreased in the CA3 subfield (292). Moreover, in the severely sclerotic Ammon's horn subfields of MTLE patients, beta1 and beta2 immunoreactivity was enhanced in some of the remaining neuronal cell bodies (293), indicating that Ca_v auxiliary beta subunits are also altered in MTLE.

5.4. Cyclic nucleotide-regulated channels

The cyclic nucleotide-regulated channel family comprises two groups of ion channels, the cyclic nucleotide-gated (CNG) and the hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels. In this review we will focus in the HCN channels, since they are widely distributed in the central nervous system, and an increasing number of studies have mechanistically linked changes in the expression of HCN channels to absence epilepsy and MTLE (199,204,294-299).

The HCN channel family comprises four members (HCN1-HCN4) (300). All HCN channels contain

six-transmembrane helices (S1–S6) and assemble in tetramers (300). The S4 segment of the channels is positively charged and serves as voltage sensor, while the C terminus contains a cyclic nucleotide binding domain that confers regulation by the cyclic nucleotides cGMP and cAMP (300). Interestingly, the stimulatory effect of cyclic nucleotides does not depend on protein phosphorylation but is caused by direct interaction with the HCN channel protein (300).

The current produced by HCN channels, commonly termed I_h , is found in a variety of excitable cells, including neurons, cardiac pacemaker cells, and photoreceptors (300,301). In neurons, I_h is critically involved in modulation of excitability by determining resting potential and generating “pacemaker potentials” (300–302). The typical features of I_h include activation by membrane hyperpolarization; permeation of Na^+ and K^+ ; and positive shift of voltage dependence of channel activation by direct binding of cyclic nucleotides (300,301).

Interestingly, both decreases and increases in HCN channel expression have been shown to occur in multiple animal models of MTLE as well as epileptic humans. In fact, mRNA and protein levels for the HCN1 channel subtype were found to be decreased in rat CA1 hippocampal subfield in the pilocarpine as well as in the kainate model of MTLE (199,204,303). On the other hand, increases in mRNA and protein levels for HCN1 have been shown in the human epileptic DG and also in the DG of pilocarpine epileptic rats (294). Regarding changes in the HCN2 channel subtype, it has been shown that mRNA for but not protein levels of this channel were decreased in the rat CA1 hippocampal subfield in pilocarpine-epileptic rats (204). In addition, mRNA levels for HCN2 were decreased in CA1 area and DG in the hippocampus of kainate-epileptic rats (295). However, no changes in HCN2 mRNA or protein levels have been found in the rat DG in the pilocarpine model as well as in the same area in the hippocampus of MTLE patients (294). Moreover, in the amygdala kindling model of MTLE, no changes in mRNA and protein levels for HCN1 or HCN2 have been found in rat hippocampal CA1 subfield or DG (295).

Interestingly, enhanced neuronal excitability during kainate-induced MTLE in the entorhinal layer III is accompanied by a decrease in the I_h (304). Moreover, a down-regulation in I_h has been also demonstrated in the hippocampus of pilocarpine-epileptic animals (199,204). These results are surprising because I_h depolarizes the resting membrane potential (300–302) and, hence, a decline in I_h might be expected to reduce excitability. However, I_h inhibition has been suggested to enhance pyramidal cell dendritic excitability by increasing the availability of Ca^{2+} channels (305), as well as by amplifying the membrane resistance (306,307). In this context, it is important to point out that I_h blockers, such as ZD7288 and CsCl have been shown to decrease electrically induced paroxysmal discharges *in vivo*, suggesting antiepileptic effects for compounds that decrease the I_h (308).

6. SUMMARY AND PERSPECTIVES

A wealth of supporting evidence indicate that the limbic system exhibits a low threshold for developing acute and chronic epileptiform activity, in some cases eventually leading to a state of enhanced excitability as in MTLE. Epileptiform activity in general is characterized by evoked and spontaneous PDS, burst of action potentials and associated poly-spiking activity in extracellular recordings. These electrophysiological abnormalities have been largely considered as the electrophysiological landmarks of interictal spikes in epilepsy. Despite stereotyped patterns of epileptiform activity, the pathogenic mechanisms leading to hyperexcitability are diverse and highly complex in acquired or symptomatic epilepsy. In contrast to genetic channelopathies, in which a mutation of a specific ion channel or receptor is thought to contribute to the epileptic phenotype, acquired epilepsy as MTLE is characterized by numerous, in most cases concurrent, alterations in the expression and function of ion channels and receptors. Furthermore, the precise combinations of abnormalities leading to more aggressive epileptic phenotypes or pharmacoresistance remain to be elucidated. Detection of these molecular and functional deficits in human epilepsy may play a predictive key role in assessing the pathogenesis of the disease in certain populations of patients. Accordingly, detection of these molecular abnormalities by non-invasive or minimally invasive assays may allow the development of tests to investigate predictive biomarkers of progression, intractability and prognosis in acquired epilepsies of difficult control as MTLE.

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