

Fang Zheng

Abstract

Accumulating evidence suggest that TRPC channels play critical roles in various aspects of epileptogenesis. TRPC1/4 channels are major contributors to nonsynaptically derived epileptiform burst firing in the CA1 and the lateral septum. TRPC7 channels play a critical role in synaptically derived epileptiform burst firing. The reduction of spontaneous epileptiform bursting in the CA3 is correlated to a reduction in pilocarpine-induced SE in vivo in TRPC7 knockout mice. TRPC channels are also significant contributors to SE-induced neuronal cell death. Although the pilocarpine-induced SE itself is not significantly reduced, the SE-induced neuronal cell death is significantly reduced in the CA1 and the lateral septum, indicating that TRPC1/4 channels directly contribute to SE-induced neuronal cell death. Genetic ablation of TRPC5 also reduces SE-induced neuronal cell death in the CA1 and CA3 areas of the hippocampus.

Keywords

Seizures • Interictal spikes • Epileptiform bursting • Canonical transient receptor potential channels

Epilepsy is a group of neurological disorders characterized by recurrent, usually unprovoked seizures. Epilepsy is one of the most common brain disorders, affecting approximately 50 million people (i.e., 1% of the general population)

globally [14]. In the United States, epilepsy is the fourth most common neurological diseases [24], and one in 26 people will develop epilepsy during their lifetime [23]. Although epilepsy can be successfully treated with antiepileptic drugs (AEDs), one third of patients suffer from refractory (i.e., drug-resistant) seizures. Therefore, there is a pressing need for the development of new anti-epileptic treatment options.

Historically, references to epileptic seizures can be found in many ancient cultures. The

F. Zheng (✉)
Department of Pharmacology and Toxicology,
College of Medicine, University of Arkansas for
Medical Science, Little Rock, AR 72205, USA
e-mail: zhengfang@uams.edu

earliest description of epilepsy has been found in a Babylonian medical textbook, and it provides a remarkably accurate account of clinical manifestations of seizures [56]. Descriptions of seizure phenomena include generalized convulsions, partial motor seizures, and sensory symptoms indicative of an aura and repetitive occurrence as in *status epilepticus*. These clinical phenomena are today recognized as parts of a broad category of symptom complexes designated as “epileptic” first by Jackson in the nineteenth century [25]. The greatest contribution by Jackson is his conceptualization of a single mechanism capable of explaining the full spectrum of epileptic phenomena. According to Jackson, all the phenomena of epileptic fits may be explained by a discharge of gray matter: focal discharges of sensory areas may lead to aura, whereas focal discharges of motor areas cause contralateral convulsion; intensification and spread of focal discharges can lead to unconsciousness and convulsion. The understanding and treatment of seizures and epilepsy have advanced greatly in the twentieth century after the discovery of electroencephalography (EEG) and its application as a critical clinical tool. EEG findings have redefined epilepsy in modern terms [20]. There are distinct EEG ictal patterns for petit mal, grand mal, and psychomotor seizures. These human EEG findings form the foundation of modern understanding of the pathophysiology of epilepsy.

The rapid development of molecular biology and human genetics has revolutionized the epilepsy field in the last 20 years. In many cases, idiopathic seizures are “channelopathies” in nature, i.e., caused by mutations that cause malfunction of voltage-gated or ligand-gated ion channels [19, 34, 46]. The current list of voltage-gated ion channels associated with seizures includes sodium channels, calcium channels, potassium channels, and chloride channels. The current list of ligand-gated ion channels associated with seizures includes GABA-A receptors, nicotinic receptors, and NMDA receptors. And the list will continue to grow in the future. The number of ion channel families involved in idiopathic epilepsy syndromes indicates a complexity in the underlying pathogenic processes for

epilepsy and presents a challenge for the rational design of the next generation of antiepileptic drugs. On the other hand, the identification of these ion channels also offers numerous clues for the understanding of the pathophysiology of epilepsy.

There are two essential epileptogenic factors that are required for the occurrence of a seizure [33, 34]. The first is the neuronal hyperexcitability that arises from cellular mechanisms that affect ion channels involved in the modulation of neuronal firing patterns. The second is a network abnormality that results in uncontrolled synchronization of large groups of neurons and propagation of the epileptic discharge along the neural pathways. Canonical transient receptor potential (TRPC) channels contribute to the occurrence of seizures by playing a role in both sets of disturbances. In this chapter, we will first review the current evidence regarding the role of various TRPC channels in the generation of epileptiform burst firing, followed by a discussion about the role of TRPC channels in synaptic plasticity and abnormal synchronization. Finally, the implication of these distinct mechanisms on seizure generation and propagation will be discussed.

11.1 Ictal vs Interictal Activities: From Clinics to Bench Side

The *International League Against Epilepsy* (ILAE) defined a seizure as “a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain” [17]. Clinically, physicians rely on EEG recording to identify, classify, and localize seizures. Abnormal EEG patterns are broadly defined as ictal (i.e., during seizures) or interictal (i.e., between seizures). However, the line between interictal and ictal epileptiform discharges can be ambiguous [16]. Practically, interictal epileptiform discharges are defined as paroxysmal activities lasting up to 200 ms, with those lasting 30–70 ms defined as “spikes” and those lasting 70–200 ms defined as “sharp waves.” This classification based on duration is

largely arbitrary and without clear clinical utility. Ictal epileptiform discharges are also complex and somewhat ambiguous. Absence seizures (“petit mal”) are associated with 3 Hz rhythmic spike and wave patterns, whereas generalized tonic-clonic seizures (“grand mal”) are characterized by 10 Hz spikes (during the tonic phase and 10 Hz spikes mixed with slow waves during the clonic phase). Focal seizures are associated with localized ictal activities and interictal activities outside of the seizure foci.

Animal studies of seizures have been largely confined to generalize seizures because of the difficulty of detecting focal seizures either electrophysiologically or behaviorally. The kindling model [35] utilizes repetitive electric stimulation of vulnerable brain structures to induce seizures, and the Racine scale [44] has been developed to describe the behavioral manifestation of seizures resulting from kindling of amygdala in rats. However, the kindling process does not result in spontaneous recurrent seizures. On the other hand, pilocarpine, a muscarinic agonist, induces *status epilepticus* (SE) acutely, and spontaneous recurrent seizures later on after the reorganization of neural network resulted from SE-induced neurodegeneration [10, 54]. Although intrahippocampal injection of kainic acid also can result in spontaneous recurrent seizures in about a third of mice, the pilocarpine model remains the mostly utilized animal model of epilepsy today. If the definition of seizures in the clinic is difficult and somewhat ambivalent, the definition of seizures in animal studies is even more challenging. The reason is simple: clinical diagnosis of epileptic seizures relies heavily on the report of symptoms and histories by patients, whereas it is difficult to discern and distinguish normal EEG patterns or behaviors from epileptic ones in animals. Although EEG recording technique has been used in animal epilepsy research, the Racine scale which describes a set of convulsive behaviors still plays a dominant role. This state of affairs has implications for the understanding of TRPC channel’s role in seizure and epilepsy and will be discussed in more details later in this chapter.

The cellular correlates of interictal and ictal activities are first investigated by Matsumoto and Marsan in 1964 [30, 31]. The spikes, the simplest epileptiform interictal discharges in EEG, are correlated with a burst of action potentials with a large underlying depolarization called paroxysmal depolarization shift (PDS) [30]. Ayala et al. first proposed that interictal spikes are generated by altered network excitability and the PDS, i.e., a plateau potential, are synaptic in origin [4]. This view gained further support by the work of Johnston and Brown [26]. Later, a competing view that explains the PDS as synchronized firing of neurons with intrinsic bursting emerged [50]. Today, it is generally accepted that epileptiform bursting can be either synaptically derived or nonsynaptically derived, depending on the specific neuronal population and neural network properties [33].

11.2 TRPC Channel’s Contribution to Intrinsic Epileptiform Burst Firing

Neuronal excitability is controlled by ion channels expressed in a given neuron. The information in the nervous system is encoded by action potentials. The firing pattern of a neuron is generally determined by its intrinsic properties, i.e., independent of synaptic inputs. An action potential is generated when the membrane potential is depolarized to a threshold point, when self-sustained activation of voltage-gated sodium channels is triggered. The rapid and large depolarization caused by this inward current carried by voltage-gated sodium channels then activates voltage-gated potassium channels, which repolarize the neuron quickly. In most cortical pyramidal neurons, the firing rate is rather limited. This is largely due to two factors: (a) the time needed to remove inactivation of voltage-gated sodium channels after each action potential and (b) the hyperpolarization before and after action potentials mediated by various types of voltage-gated potassium channels and non-voltage-gated potassium channels [9, 21]. The A-type potassium channels put break on the membrane

depolarization, slowing down the triggering of the next action potential [12], whereas potassium channels responsible for the afterhyperpolarization delay the return to the resting membrane potential. One additional layer of control is the phenomenon known as “spike adaptation,” i.e., a gradual decrease in action potential frequency during sustained membrane depolarization, which is mediated by M-type potassium channels [22] or the HERG-type potassium channels [55]. Dentate granule cells show similar AHP and spike adaptation as pyramidal neurons [47]. In contrast, interneurons have a higher firing frequency because they can recover more quickly from inactivation of voltage-gated sodium channels and they have less afterhyperpolarization. Thus, glutamatergic principal neurons typically fire at low frequency and exhibit strong spike adaptation, and there are built-in safeguards against hyper-excitability.

However, some pyramidal cells exhibit bursting behavior, i.e., a train of action potentials sustained by a slow membrane depolarization lasting 100–400 ms [28]. It should also be noted that the firing pattern of a pyramidal neurons is malleable and subjected to neuromodulation. For examples, activation of group I metabotropic glutamate receptors (mGluRs) can elicit burst firing in CA1 pyramidal neurons [11] or lateral septal neurons (which are GABAergic) [61]. Activation of muscarinic receptors by carbachol has similar effects in many cortical neurons. Synaptic plasticity also can alter the firing pattern of pyramidal neurons. The best known example is the CA3 pyramidal neuron, in which the activity-dependent long-term potentiation of recurrent collateral synapses leads to synchronized bursting of a large group of CA3 pyramidal neurons that resembles epileptiform discharges [2, 48].

The ion channels responsible for the slow depolarization underlying burst firing include low-threshold voltage-gated calcium channels (i.e., the T-type; $Ca_v3.1-3.3$) [3, 7] and hyperpolarization-activated cyclic nucleotide-gated (HCN) cation channels (i.e., the H-current or the Q-current; HCN1–4) [21, 27]. In addition

to these voltage-gated ion channels, the so-called calcium-activated nonselective (CAN) cation channels are also known to contribute to the slow depolarization driving the bursting. The molecular identity of the CAN channels had been a mystery for many years, and recent studies revealed that they are largely heteromeric TRPC channels comprised of TRPC1 and TRPC4.

The activation of TRPC channels by mGluRs and its implication in the generation of epileptiform discharges has been thoroughly investigated in the lateral septal neurons. The lateral septal nucleus is an important relay nucleus in the limbic system, which is an integral part of the septohippocampal loop: (1) The lateral septal nucleus receives its excitatory input primarily from the CA3 region of the hippocampus; (2) lateral septal neurons project to the medial septal nucleus and the diagonal band of Broca; and (3) the neurons in the medial septum and the diagonal band of Broca project back to the hippocampus to complete the loop [18]. The lateral septal nucleus also receives prominent serotonin input from the dorsal raphe nucleus, norepinephrine input from the locus coeruleus, and dopamine input from the ventral tegmental area and has reciprocal connections with the hypothalamus. Thus, the firing of lateral septal neurons is modulated by essentially every neurotransmitter or neuromodulator in the central nervous system. Interestingly, the effects of most neurotransmitters or neuromodulators are inhibitory, i.e., causing membrane hyperpolarization and reduced firing. Only agonists for group I mGluRs and m1 muscarinic receptors are excitatory, i.e., causing membrane depolarization and bursting [18]. The ion channels responsible for epileptiform bursting induced by mGluR agonists are initially characterized as CAN channels because they are clearly calcium-dependent nonselective cation channels [61–63]. However, they exhibit a very strong negative slope region in their current-voltage relationship, a rather peculiar property for non-voltage-gated ion channels [45]. These channels have now been identified convincingly as TRPC1/4 channels because genetic ablation of either TRPC1 or TRPC4 is sufficient

to abolish the plateau potential underlying epileptiform bursting induced by group I mGluR agonists [40]. Furthermore, genetic ablation of TRPC3, 5, 6, and 7 has no detectable effects [64]. It remains unclear whether the excitatory effects of carbachol in lateral septal neurons are also mediated by TRPC1/4 channels, since carbachol has yet to be tested in any TRPC knockout mice.

Activation of group I mGluRs also elicits epileptiform burst firing in CA1 pyramidal neurons, and these bursts are also dependent on heteromeric TRPC1/4 channels [42]. The epileptiform bursting in the CA1 pyramidal neurons can also be elicited by carbachol, a muscarinic agonist. The amplitude of the plateau underlying the epileptiform bursts in CA1 pyramidal neurons is significantly reduced in TRPC1KO and TRPC1/4DKO mice [42]. The number of spikes in each burst is also significantly reduced in TRPC1KO and TRPC1/4DKO mice [42]. These findings are similar to what are observed in the lateral septum, suggesting that the activation of heteromeric TRPC1/4 channels by group I mGluRs plays a critical role in the epileptiform bursting. TRPC1 and TRPC4 are not the only TRPC subfamily members expressed in CA1 pyramidal neurons. TRPC5 is also highly expressed in CA1 pyramidal neurons. A previous study [49] has reported that carbachol induces membrane insertion of TRPC5 channels in CA1 pyramidal neurons and postulated that TRPC5 channels are responsible for the epileptiform bursting induced by muscarinic agonists. Strikingly, genetic ablation of TRPC5 has no detectable effects on mGluR agonist-induced epileptiform bursting in CA1 pyramidal neurons [42]. This is really surprising given that TRPC4 and TRPC5 are highly homologous and are thought to be able to form heteromeric channels with each other and with TRPC1. These results indicate that TRPC4 and TRPC5 have distinct functional roles even though they may be expressed in the same neuron.

Due to a lack of specific antibodies for many members of the TRPC subfamily, it is unclear whether CA1 pyramidal neurons also express

other TRPC subfamily members. A previous study reported that TRPC3 channels are required for BDNF-induced cation current and dendritic spine formation [1]. However, genetic ablation of TRPC3 channels has no detectable effects on mGluR agonist-induced epileptiform bursting in CA1 pyramidal neurons (personal observation; Phelan et al.). This is clearly opposite to the reported role of TRPC3 channels in immature cortex [65]. Epileptiform bursting induced by mGluR agonists is also unaltered in TRPC7KO mice [41]. Thus, the evidence so far indicates that participation in epileptiform bursting is a distinct role of heteromeric TRPC1/4 channels in at least two brain regions.

Can TRPC5 channels contribute to bursting in other cortical regions? Yan et al. reported that transfection of dominant negative TRPC5 into pyramidal neurons in cortical slice cultures abolishes carbachol-induced slow afterdepolarization, whereas overexpression of TRPC5 has the opposite effects [58]. These observations have been interpreted as TRPC channels being required for the carbachol-induced slow afterdepolarization in these neurons. However, the exact subunit composition remains unclear. Further studies are required to determine exactly which type of TRPC channels are responsible for the carbachol-induced slow afterdepolarization and burst firing in many cortical areas.

Although epileptiform bursting induced by mGluR agonists or muscarinic agonists are potential cellular correlates of seizure activities, it must be noted that permanent activation of TRPC channels in the absence of these agonists has to occur if they contribute to the generation of spontaneous recurrent seizures. There are reports of upregulation of some TRPC channels in human patients suffering from seizures [57, 60]. However, it remains unclear whether such changes occur in animal models of seizure and epilepsy. Furthermore, it remains unclear whether the upregulation of these TRPC channels is also associated with spontaneous epileptiform burst firing. These questions need to be answered by future studies.

11.3 Contribution to Synaptically Derived Epileptiform Burst Firing by TRPC Channels

In addition to changes of intrinsic membrane properties, changes of synaptic signaling strength can also contribute to the generation of epileptiform burst firing. Dingledine et al. reported that blocking GABAergic inhibition by bicuculline triggers epileptiform burst firing in CA1 pyramidal neurons, and these bursts (either evoked by stimulating Schaffer collateral or spontaneous) are reduced by AP5, a selective NMDA receptor antagonist [15]. However, it appears that the role of NMDA receptors is different in CA3 pyramidal neurons [2]. High-frequency stimulation of recurrent collaterals induces epileptiform bursting in CA3 pyramidal neurons, and AP5 can block it when applied before the high-frequency stimulation but is ineffective when applied after the induction of epileptiform bursting. Further studies showed that the epileptiform bursting in CA3 pyramidal neurons can also be elicited by bathing the slice in high K^+ extracellular solutions [5] or the GABA-A receptor antagonist bicuculline [48]. No matter how the bursting is elicited, it has been shown consistently that the bursting results from an activity-dependent long-lasting increase in synaptic strength at CA3 recurrent synapses, which recruits a group of CA3 pyramidal neurons to fire action potentials synchronously. This long-term potentiation of the CA3 recurrent synapses depends on the activation of both NMDA receptors and group I mGluRs. The most striking property of synaptically derived epileptiform bursting in CA3 pyramidal neurons is its self-sustaining nature. Once induced, these bursting can last for hours after the washout of high K^+ or bicuculline. This is in stark contrast to the epileptiform bursting in CA1 pyramidal neurons induced by muscarinic agonist or mGluR agonists, which is reversible upon washout of these agonists. Furthermore, the spontaneous epileptiform bursting in CA1 provides the drive for epileptiform bursting in entorhinal cortex because severing the connection between the two areas abolishes the bursting in entorhinal cortex but leaves the bursting in CA3 intact. For

these reasons, the epileptiform bursting resulting from the LTP of RC synapses is the most convincing model of epileptogenesis *in vitro*.

Given the evidence that the involvement of mGluRs in the generation of epileptiform burst firing in CA3 pyramidal neurons, TRPC channels, which are coupled to mGluRs, are also likely involved. TRPC1 and TRPC4 are potential candidates because of the critical role of TRPC1/4 channels in the epileptiform bursting in CA1 pyramidal neurons. However, the spontaneous burst firing induced by bicuculline in CA3 pyramidal neurons is unaltered in TRPC1KO mice or TRPC1/4DKO mice, suggesting that TRPC1 and TRPC4 do not play a critical role in the generation epileptiform bursting in CA3 pyramidal neurons (personal communication, Phelan et al.). TRPC3 is another likely candidate because of its critical role in the BDNF-TrkB signaling pathway. Genetic ablation of TRPC3 also has no discernable effects on spontaneous epileptiform bursting in CA3 pyramidal neurons (personal communication, Phelan et al.). Surprisingly, TRPC7, a little known member of the TRPC subfamily, turns out to play a critical role in spontaneous epileptiform bursting in CA3 pyramidal neurons [41].

TRPC7 is the last member of the TRPC subfamily to be cloned and is highly expressed in the brain [38]. However, the distribution of TRPC7 mRNA is rather low in the mouse Allen Brain Atlas, with its highest expression level detected in the cortical subplate. The protein expression pattern of TRPC7 is unclear due to a lack of a specific antibody. TRPC7 shows a high degree of sequence homology to TRPC3 and TRPC6, and these three members of the TRPC subfamily belong to a subgroup that can be directly activated by diacylglycerol (DAG). Genetic ablation of TRPC7 significantly reduced spontaneous epileptiform bursting in CA3 pyramidal neurons. The duration of the self-sustained bursts (i.e., 20 min after washout of bicuculline) was reduced from 197 ms down to 97 ms, and the amplitude of the plateau underlying the burst was reduced from 36 mV down to 24 mV. The reduction of epileptiform bursting is associated with a reduction of LTP at CA3 recurrent synapses, whereas

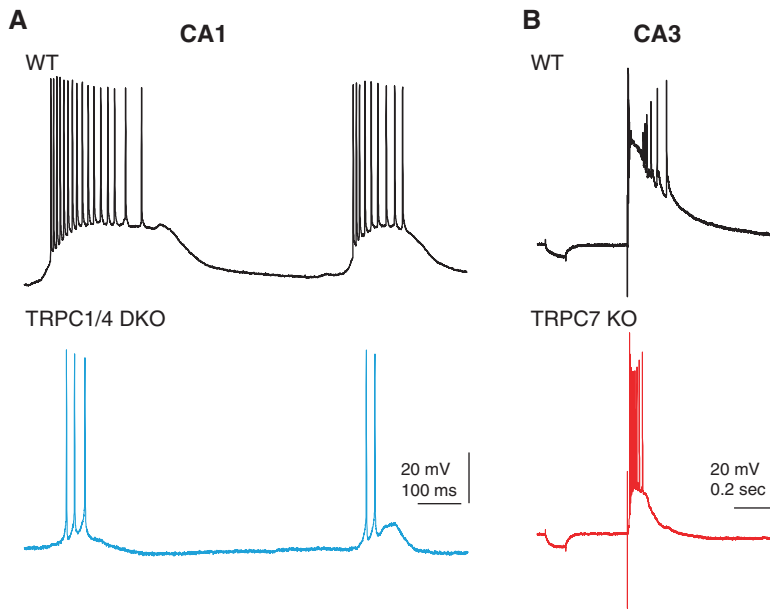


Fig. 11.1 Contribution of TRPC channels to epileptiform burst firing in the hippocampus. (a) The spontaneous epileptiform burst firing in CA1 pyramidal neurons shown is induced by 30 M 1S,3R-ACPD, a mGluR agonist. TRPC1/4 channels are required for this epileptiform bursting. (b) Epileptiform burst firing in CA3 pyramidal

neurons is evoked by mossy fiber stimulation after bath application of 20 M bicuculline for 30 min. The persistent bursting results from activity-dependent strengthening of recurrent collaterals. TRPC7 channels play a critical role in this process (Adapted with permission from Refs. [41, 42])

the LTP at mossy fiber synapses is normal in TRPC7KO mice. Interestingly, the Schaffer collateral LTP in the CA1 is also reduced in TRPC7KO mice. Since Schaffer collaterals and recurrent collaterals share the same origin, these findings suggest that TRPC7 channels are likely located on the presynaptic terminals of both Schaffer collaterals and CA3 recurrent collaterals and are involved in the modulation of LTP through presynaptic mechanisms. What are the ion channels responsible for the plateau potential underlying spontaneous epileptiform bursting in CA3 pyramidal neurons? It has been reported that although the induction of bursting requires functional NMDA receptors, NMDA antagonists have no effects on the amplitude or the duration of the bursts. Therefore, NMDA receptors contribute little to the plateau potential underlying epileptiform bursting in CA3 pyramidal neurons. It is possible that TRPC channels contribute significantly to the plateau potential (Fig. 11.1), but this cannot be tested directly yet because of the

lack of drugs that can selectively block specific subtypes of TRPC channels.

11.4 How Do the In Vitro Findings Correlate with Acute Seizures In Vivo?

The potential role of TRPC channels in in vivo seizures has just begun to be investigated using the pilocarpine model. The recent reports are limited to the role of TRPC channels in pilocarpine-induced SE and SE-induced neurodegeneration. The role of TRPC channels in the SE-induced spontaneous recurrent seizures has yet to be determined. Before a detailed discussion about the published data regarding any TRPC channel's role, it is critical to thoroughly discuss the pilocarpine model itself, the Racine scale, and other parameters frequently used to assess the role of a given molecular target.

Pilocarpine is an agonist for M1 subtype of muscarinic receptors, and administration of

pilocarpine in rodents results in status epilepticus in a dose-dependent manner. This dose-response curve is a rather steep one [32, 37], and the lower dose of pilocarpine (40 mg/kg for mice) actually has anticonvulsant effects in the maximal electric shock (MES) model of seizures [59]. The initial site of action is the hippocampus, because the seizure activities in the hippocampus precede seizure activities in the cortex and the thalamus [54]. EEG recording in both rats and mice revealed a long latent period before the appearance of cortical seizures [43, 52]. This latent period is characterized by a suppression of normal cortical EEG activity, but a progression from immobility to forelimb clonus and Straub's tail [40, 43]. A burst of cortical seizures appears after a substantial delay (20–40 min following the administration of pilocarpine) and is followed by a postictal depression in the EEG signals but not behaviorally [43]. This process repeats several times with the length of the seizure increasing each time, until the SE state is finally reached [41, 43]. Most Racine stage 4 and 5 convulsive behaviors occur during this transition period, and once the SE is established, only stage 3 or lower convulsive behaviors remain during the remaining period of SE which lasts hours [43]. At lower doses, pilocarpine either only causes short bursts of epileptiform discharges in cortical EEG that can be characterized as interictal activities or only depression of normal EEG activities (personal communication, Zheng).

The Racine scale remains to be widely used in animal studies of seizure and epilepsy and is regarded by many as an indicator of seizure severity. This widely accepted notion has been recently challenged by quantitative EEG analysis of pilocarpine-induced seizures in mice [43]. With the exception of the transition period, the Racine scores do not correlate to the RMS power of cortical EEG signals. These results support previous reports that the Racine scale describes the involvement of distinct brain areas (or circuitry), rather than increasing intensity of seizures. Thus, a reduction of Racine scores does not necessarily indicate a reduction in cortical seizure severity or intensity. Another frequently used parameter in animal seizure research is the

latency to the onset of seizures. The peculiar characteristic of the latency to pilocarpine-induced SE is that it is not dose-dependent (Personal communication, Zheng). The duration of the transition period is also fixed, with very little animal to animal variations. It is difficult at the moment to ascertain the pathophysiological implication of a change in the latency to SE.

Initial work on the role of TRPC channels in pilocarpine-induced acute seizures relied solely on the behavioral manifestation of seizures graded using the Racine scale [40, 42]. Using this approach, the pilocarpine-induced acute seizures in TRPC1 knockout mice or TRPC1/4 double knockout mice are comparable to the pilocarpine-induced acute seizures in wild-type mice. Since heteromeric TRPC1/4 channels are critical for epileptiform burst firings in the lateral septum and the CA1 area of the hippocampus, these results are puzzling. It is likely a reflection of the limited discriminating power of the behavioral approach. The EEG approach needs to be utilized to conclusively determine the role of TRPC1 and TRPC4 in pilocarpine-induced acute seizures.

Using the same behavioral approach, it has been reported that TRPC5 knockout mice exhibited reduced Racine scores during the late phase, but not the early phase of pilocarpine-induced SE [42]. One possible explanation for this observed change is the reduced Schaffer collateral LTP in TRPC5 knockout mice. In *in vitro* experiments in slices with intact hippocampal-entorhinal circuitry, the ictal activities are often initiated in the CA1 area, driven by epileptiform bursting in CA3 pyramidal neurons [6, 39, 51]. Therefore, a reduction of Schaffer collateral LTP is expected to hinder the hyper-excitability of the CA1 area. However, it remains unclear whether the reduced Racine score during the late phase of pilocarpine-induced SE truly reflects a reduction of cortical seizure intensity without confirmation from EEG analysis.

The TRPC channel associated with the most striking role in pilocarpine-induced SE is surprisingly the least studied TRPC7. Genetic ablation of TRPC7 drastically reduced the occurrence of pilocarpine-induced SE. Pilocarpine, at 280 mg/kg, induced SE in 10 out of 11 wild-type mice.

The same dosage of pilocarpine induced only a suppression of cortical EEG in a majority of TRPC7 knockout mice [41]. A detailed power spectral analysis indicates that there was a selective change in gamma wave activities in TRPC7 knockout mice [41]. Gamma wave activities normally increase significantly during the silent period after the administration of pilocarpine, and this increase precedes the occurrence of generalized cortical seizures that marks the beginning of the transition period and is correlated moderately to the Racine scores [43]. Genetic ablation of TRPC7 selectively abolishes this pilocarpine-induced increase in gamma wave activities during the latent period. These findings indicate that the pilocarpine-induced increase in gamma wave activities likely plays a critical role in the spread of pilocarpine-induced seizures from the hippocampus to the cortex. There are two possible gamma wave generators in the hippocampus: (1) the dentate gyrus and (2) the CA3 area [13]. There is a strong possibility that pilocarpine directly acts in the CA3 to produce convulsive behaviors and increased cortical gamma wave activities, because multiple studies have reported pilocarpine-induced interictal and ictal-like activities in the CA3 [6, 54].

11.5 The Contribution of TRPC Channels to SE-Induced Neurodegeneration

SE-induced neurodegeneration is a critical element in the pilocarpine model of epilepsy [10, 36, 53]. It is commonly accepted that SE-induced hippocampal neuronal cell death is a prerequisite to the epileptogenesis. In other words, it is believed that without SE-induced neurodegeneration, spontaneous recurrent seizures will not occur later on.

It has been reported that there appears to be a threshold for the “severity” of pilocarpine-induced seizures to induce neurodegeneration. Only mice that experienced seizures greater than Racine stage 3 show seizure-induced neuronal cell death [8, 40]. This threshold can be viewed simply as the presence of cortical SE, since it has

been reported Racine stage 4 and 5 convulsions occur during the transition to SE [43]. There appears also to be a minimal duration of SE for resulting in neuronal cell death [32]. The general pattern of SE-induced neuronal cell death is also relatively consistent across multiple species [53]. Typical vulnerable brain regions include the hilar regions, CA1 and CA3 regions of the hippocampus, limbic regions such as amygdala and lateral septum, thalamus, hypothalamus, entorhinal cortex, and cingulate cortex. Interestingly, the dentate granule cells and CA2 hippocampal pyramidal cells are typically resistant to SE-induced cell death.

Changes in SE-induced neuronal cell death occur in various TRPC knockout mice. SE-induced neuronal cell death is reduced in TRPC1/4 double knockout mice but not in TRPC1 knockout mice or in TRPC5 knockout mice. The roles of TRPC3 and TRPC6 are suggested to be opposite: blocking TRPC3 channels by Pyr3 and activating TRPC6 channels by hyperforin both reduce pilocarpine-induced neuronal cell death [29]. However, these proposed roles are not supported by data from TRPC3 or TRPC6 knockout mice (Phelan et al., personal communication). The changes in SE-induced neuronal cell death by genetic ablation or pharmacological blockade of various TRPC channels raise several questions regarding the commonly accepted notions about the excitotoxicity and indicate that the underlying molecular mechanisms for seizure-induced neurodegeneration are not fully understood. On the one hand, neuronal cell death can be prevented without any significant changes in SE severity or duration. This occurs in TRPC1/4 double knockout mice. As discussed earlier, TRPC1/4 double knockout mice clearly exhibit unmitigated pilocarpine-induced SE but significantly reduced neuronal cell death in the CA1 area as well as lateral septum. This finding suggests that TRPC1/4 channels are directly involved in the signaling cascade leading to neuronal cell death (Fig. 11.2). However, the details of the signaling downstream of TRPC1/4 channel activation remain unexplored. On the other hand, unpublished data indicates that neuronal cell death can occur without

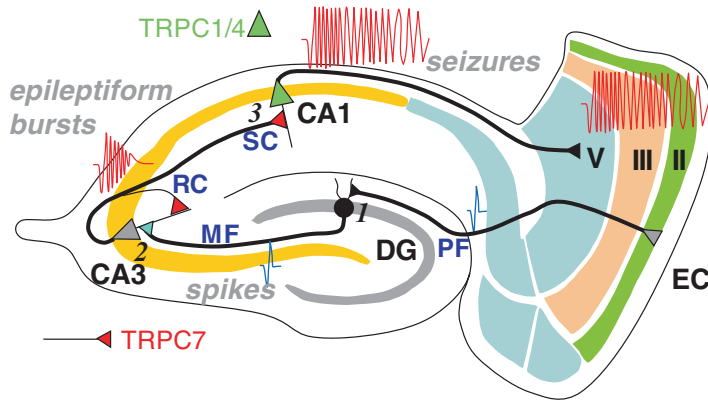


Fig. 11.2 The generation of seizures in the entorhinal-hippocampal loop. We propose that the generation of synaptically derived epileptiform bursting in CA3 requires TRPC7 channels. The epileptiform bursting in the CA3

drives the generation of ictal-like activities in the CA1, which then propagate to the entorhinal cortex. TRPC1/4 channels contribute to seizure-induced neuronal cell death in the CA1 (Adapted with permission from Ref. [41])

sustained SE in a TRPC knockout line (personal communication, Phelan et al.). This appears to support the notion that some TRPC subfamily members may play a neuroprotective role. Furthermore, the anatomical pattern of SE-induced neurodegeneration is also altered in some TRPC knockout lines. All these issues need to be taken into account for the future study of the TRPC channel's role in epileptogenesis using the pilocarpine model.

11.6 Perspectives

The critical roles of various TRPC channels in epileptiform bursting in brain slices suggest that these channels likely play a significant role in the generation of spontaneous recurrent seizures in vivo. Hopefully, future studies will provide direct evidence that will elucidate the precise role of each TRPC subfamily member in epileptogenesis. TRPC channels have great potentials as novel molecular targets for the development of the next generation of antiepileptic drugs.

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