Do Interictal Spikes Sustain Seizures and Epileptogenesis?

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Interictal spiking is seen in the EEG of epileptic patients between seizures. To date, the roles played by interictal events in seizure occurrence and in epileptogenesis remain elusive. While interictal spikes may herald the onset of electrographic seizures, experimental data indicate that hippocampus-driven interictal events prevent seizure precipitation. Even less clear than the role of interictal events in seizure occurrence is whether and how interictal spikes contribute to epileptogenesis. Thus, while plastic changes within limbic neuronal networks may result from ongoing interictal activity, experimental evidence supports the view that epileptogenesis is accompanied by a decrease in hippocampus-driven interictal activity.

The EEG of patients presenting with partial seizures is characterized by brief, epileptic spikes that are not associated with evident clinical symptoms. The interictal spiking is valuable for diagnosing the epileptic condition, and when required, for localizing the epileptogenic area. Interictal and ictal discharges in animal models of epileptiform activity consist of similar (but for duration) neuronal depolarizations, leading to sustained action potential firing (1–4), suggesting that interictal and ictal events may reflect similar neuronal mechanisms. Moreover, interictal spikes may herald the onset of electrographic seizures. However, the precise relationship between interictal and ictal activity remains ambiguous, as careful studies performed in patients with temporal lobe epilepsy (TLE) and in animal models mimicking this condition indicate that the interictal spike rate does not change before seizure onset (5–7). Finally, it has been proposed that interictal spiking prevents seizure precipitation in some animal models (8–11).

Even more elusive than interictal–ictal relationship is the role played by interictal spikes in epileptogenesis, which is the process leading to the development of an epileptic condition (12). While it is indisputable that plasticity is a fundamental characteristic of neuronal networks, as epitomized by the kindling phenomenon (13), it is unclear whether changes in synaptic efficacy or formation of new connections result from ongoing interictal activity. Here, we review data indicating that interictal spikes can have both anti- and proseizure actions. In addition, experimental evidence supporting the view that a decrease in hippocampal-driven interictal activity contributes to epileptogenesis in the pilocarpine model of TLE is summarized. The hippocampus (and in particular its CA3 subfield) is the limbic area that is most prone to generate interictal events, at least with in vitro preparations (4).

Antiseizure and Proseizure Actions of Interictal Spikes

Because TLE patients present with seizure discharges in limbic structures, such as the entorhinal cortex (EC) and the hippocampus proper, several electrophysiological studies have been carried out on rodent brain slices that contain reciprocally interconnected portions of hippocampal and parahippocampal areas (4) or with isolated brain preparations (11,14,15). As illustrated in Figure 1A, under appropriate conditions (e.g., when treated with 4-aminopyridine), combined EC–hippocampus slices generate epileptiform discharges resembling interictal and ictal events (4). Interictal activity, which is caused by non-*N*-methyl-D-aspartate (NMDA) glutamatergic mechanisms, originates in the CA3 subfield of the hippocampus, spreads via the CA1/subiculum areas to the EC, and returns to CA3 via the perforant path/dentate gyrus (Figure 1Ba). In contrast, ictal events—dependent on the activation of both NMDA and non-NMDA glutamatergic and $GABA_A$ receptors—initiate in the EC and propagate to the hippocampus (Figure 1Bb). The EC is known to be prone to generate seizures in TLE patients (16–18). In vitro studies also have shown that ictal discharges disappear within 1–2 hours, while the CA3-driven interictal activity occurs throughout the experiment (Figure 1A). Moreover, cutting the Schaffer collaterals, which connect CA3 to CA1, abolishes interictal spikes in the EC and allows ictal

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Epilepsy Currents, Vol. 6, No. 6 (November/December) 2006 pp. 203–207
Blackwell Publishing, Inc.

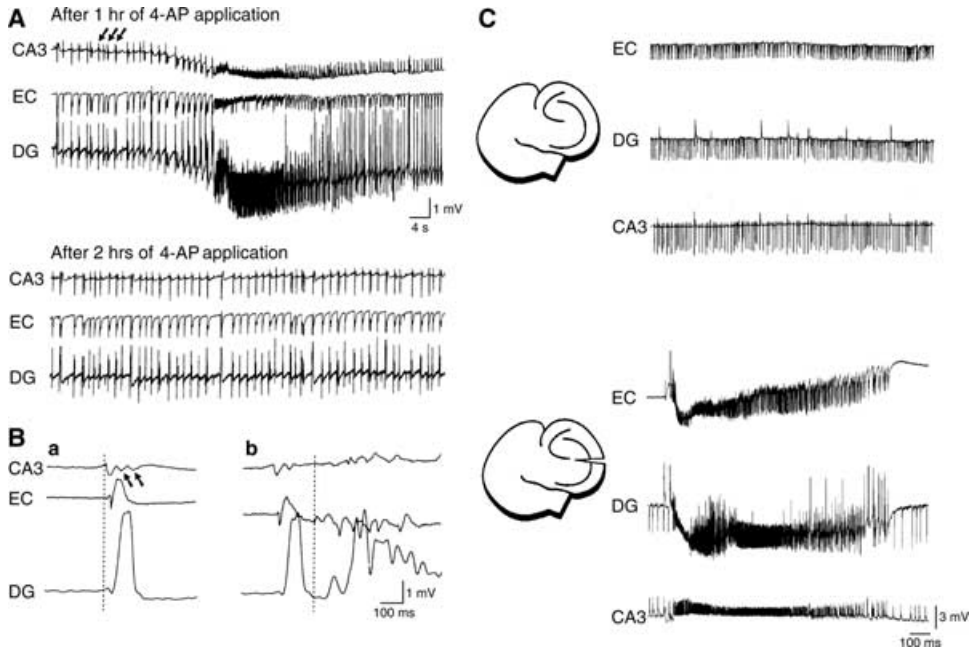

FIGURE 1. A: Spontaneous epileptiform activity recorded at hour 1 and 2 during continuous bath application of 4-AP. Simultaneous field potential recordings were made in the CA3 stratum radiatum, the deep layers of the EC, and the DG cell layer. The interictal discharges recorded at 1 hour are indicated by arrows; note that after 2 hours of 4-AP application, ictal discharges disappear. Adapted with permission from *J Neurosci.* (10) Copyright 1997 Society for Neuroscience. B: Expanded traces of interictal (**a**) and ictal discharges (**b**) induced by 4-AP (~1 hour) in an intact EC–hippocampal combined slice. In **a**, the interictal discharge initiates in the CA3 region and propagates to the EC and DG; arrows point to the late components of the interictal discharge recorded in CA3. In **b**, the ictal discharge is preceded by an interictal event, with a temporal profile similar to that seen in **a**; note, however, that the site of origin of the ictal discharge occurs in EC. Dotted lines in **a** and **b** were positioned at the time of the earliest visible deflection in the three-field potential recordings. Adapted with permission from *J Neurosci.* (10) Copyright 1997 Society for Neuroscience. C: Effect of cutting the Schaffer collaterals on 4-AP-induced epileptiform discharges. Field recordings were obtained in an intact EC–hippocampal slice after ictal discharges have stopped occurring (upper panel) and after Schaffer collateral cut (lower panel); note that this procedure makes CA3-driven interictal events disappear in EC, while ictal discharges remain unabated. Adapted with permission from *J Neurophysiol.* (46) Copyright 2000 American Physiological Society. EC, entorhinal cortex; DG, dentate granule; 4-AP, 4-aminopyridine.

discharges to be reestablished in this area (Figure 1C). Therefore, CA3-driven interictal activity can reduce, rather than sustain, the ability of the EC to generate ictal events (10).

The antiseizure action exerted by interictal activity (9,19) can be mimicked by electrical stimuli at rates that are similar to those of the CA3-driven interictal activity (i.e., approximately 1 Hz). In addition, an inverse relationship between ictal and interictal discharges occurs in hippocampal slices when using drugs (e.g., baclofen) that depress interictal spikes (20,21). Evidence from the in vitro isolated guinea pig brain, indicates that periodic interictal spiking in the piriform cortex prevents involvement of this region by seizure-like activity generated in the EC–hippocampus during transient GABAergic impairment (11). Finally, interictal spikes are known to be followed by a prolonged period of inhibition (22) in which the threshold for the generation of an epileptic discharge is increased—an effect found both in models of epileptiform activity (23) and in patients with neocortical epilepsy (24).

The role of interictal spikes, however, is not as straightforward as indicated by the results reviewed above. Early experiments performed using focal discharges induced in vivo by convulsant drugs showed that interictal discharges sometimes accelerate before the onset of seizure (1). Moreover, initial studies in the kindling model suggested that interictal spikes become more frequent as the kindling process evolves and may increase prior to the appearance of spontaneous seizures (25,26). This evidence, however, was not confirmed by continuously monitoring of the EEG in kindled animals (27). More recently, analysis of the epileptiform activity induced by 4-aminopyridine in the EC revealed that local interictal spikes, which are largely contributed by $GABA_A$ receptor-mediated conductances, lead to electrographic seizure onset (4). As illustrated in Figure 2 (A and B panels), the onset of an ictal discharge recorded intracellularly from EC neurons is characterized by a long-lasting depolarization, resembling what is seen during an interictal event generated within the EC network. The similarity between

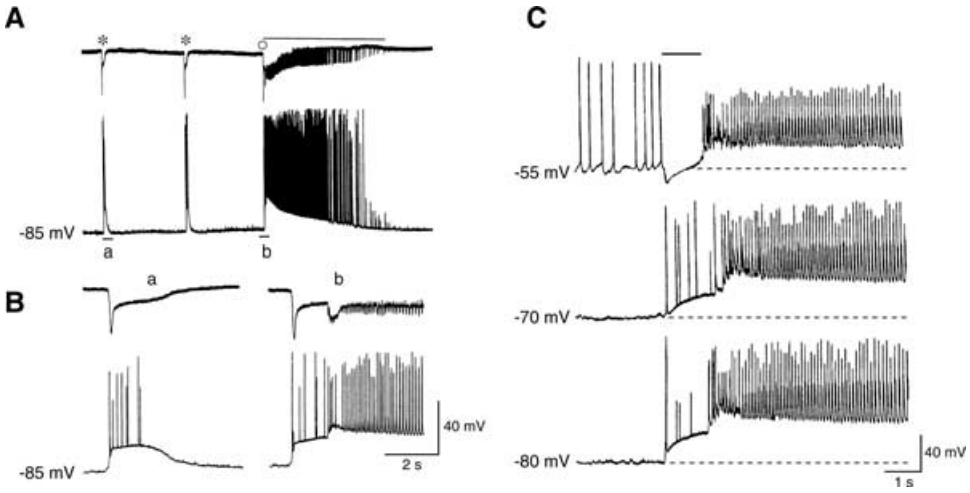

FIGURE 2. A and B: Field and intracellular (potassium-acetate–filled microelectrode) recordings from the EC demonstrate two types of activity during 4-AP application. Slow interictal and ictal discharges are identified with asterisks and an open circle, respectively. Note the similarities between the isolated interictal discharge (**Ba**) and the onset of the ictal event (**Bb**). Adapted with permission from *J Neurophysiol.* (47) Copyright 1998 American Physiological Society. C: When the neuronal membrane is depolarized by intracellular injection of steady positive current (−55 mV), the amplitude of sustained ictal depolarization decreases, while the initial long-lasting depolarization becomes hyperpolarizing as compared with the recording obtained at resting membrane potential (−70 mV). When the membrane is hyperpolarized by intracellular injection of steady negative current (−80 mV) both long-lasting depolarization and ictal depolarization increase in amplitude as compared with the samples obtained at resting membrane potential. The time occupied by this initial long-lasting depolarization is indicated by the continuous line on top of the −55 mV trace. Adapted with permission from *J Neurophysiol.* (47) Copyright 1998 American Physiological Society. EC, entorhinal cortex; DG, dentate granule; 4-AP, 4-aminopyridine.

the local interictal discharge and the event recorded at seizure onset is further supported by evidence showing that ictal discharge onset consists of a hyperpolarization when the neuron is depolarized with steady current injection (Figure 2C). Thus, in this in vitro model of limbic seizures, ictal depolarizations paradoxically originate from a hyperpolarizing event. Interictal spiking also has been observed ahead of ictal discharges in the isolated guinea pig brain preparation during short-lasting bicuculline treatment (15). Hence, interictal spikes may exert either a protective or precipitating role with respect to seizure generation.

Interictal Spikes and Epileptogenesis

Interictal spiking is the first sign of an epileptic discharge appearing after status epilepticus (SE) in animals committed to develop seizures (28–30). However, it is unknown whether this activity reflects an altered neuronal network unable to impede the ongoing epileptogenic process or a sign of incipient seizure activity. Moreover, investigators ignore whether and how interictal discharges change in their occurrence, shape, and underlying mechanisms during the period that follows SE. Indeed, after pilocarpine-induced SE, CA3-driven interictal activity in unable to control ictal discharges recorded during 4-aminopyridine treatment: electrographic seizures originating in the epileptic EC occur throughout the experiment, while they disappear in slices from nonepileptic control animals (31). In contrast, the kindling phenomenon and the development of mirror foci suggest that activity-dependent changes in synaptic transmission along with formation of new synaptic connections represent potential epileptogenic factors. In this context, recurrent interictal events could play a role in epileptogenesis, perhaps through the induction of activity-dependent synaptic plasticity mechanisms.

A point that merits critical evaluation is the assessment of mechanisms underlying the impairment of hippocampal networks generating interictal activity after SE. It is known that TLE patients display mesial temporal (or Ammon's horn) sclerosis. This condition, which is characterized by a rather selective loss of neurons in specific limbic areas (32,33), also is found in animal models of TLE (34–36). Experimental evidence suggests that in the presence of Ammon's horn sclerosis, both cell loss and the consequent synaptic reorganization contribute to epileptogenesis (37–40). In the model of self-sustaining SE, lesions are extensive in the hippocampus and CA3 pyramidal cells numbers are reduced to approximately 50% of control values (41). However, in spite of such damage, the hippocampus is still able to generate interictal spikes (29). A decrease in hippocampal network function associated with cell damage also occurs in pilocarpine-treated epileptic animals (31,4). Recurrent limbic seizures persist in this model when mossy fiber sprouting, but not neuronal damage, is reduced by protein synthesis inhibition

(42). Therefore, neuronal damage after SE may play a role in epileptogenesis.

In line with the hypothesis that neuronal damage may influence epileptogenesis following SE, cell damage and synapse loss in the CA3/CA1 areas of pilocarpine-treated animals appear to be associated with decreased control exerted on EC excitability by hippocampal output activity. Moreover, it recently has been shown that decreased hippocampal output activity in epileptic animals reflects a diminished excitatory transfer to CA3 pyramidal cells (43,44). The hypothesis has been tested with intrinsic optical signal imaging of the stimulus-induced responses in slices of pilocarpine-treated epileptic rats, demonstrating that intrinsic optical signals in CA3 were lower than in nonepileptic control slices following dentate gyrus stimulation. However, comparable responses were observed in both epileptic and nonepileptic animals when stimuli were delivered directly in CA3 (45). This decreased network-driven excitation may affect the ability of CA3 to generate effective interictal outputs that are able to control epileptiform synchronization in the EC, thus contributing to epileptogenesis.

Conclusions

The control of EC epileptiform excitability by hippocampal output activity suggests that interictal discharges may interfere with ictal events. If true, rhythmic, low-frequency stimulation of an epileptic brain may protect against seizures. This evidence, however, is at odds with what has been obtained in some models of epileptiform discharge, in which interictal events lead to electrographic seizures. The discrepancy may be explained by viewing interictal spiking as a heterogeneous phenomenon that reflects the involvement of different neuronal networks and mechanisms (e.g., synaptic conductances) in different regions of an epileptic brain. Finally, detailed studies of interictal spiking occurring after SE are required before attempting to establish its role in epileptogenesis.

Acknowledgments

This study was supported by grants from the Canadian Institutes of Health Research (CIHR; grant 8109), the Mariani Foundation (grant 06-50), the Savoy Foundation for Epilepsy, and Citizens United for Cure in Epilepsy (CURE).

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