How Does the Balance of Excitation and Inhibition Shift during Epileptogenesis?

Cell Domain-Dependent Changes in the Glutamatergic and GABAergic Drives during Epileptogenesis in the Rat CA1 Region El-Hassar L, Milh M, Wendling F, Ferrand N, Esclapez M, Bernard C. *J Physiol* 2007;578(Pt 1):193–211.

An increased ratio of the glutamatergic drive to the overall glutamatergic/GABAergic drive characterizes the chronic stage of temporal lobe epilepsy (TLE), but it is unclear whether this modification is present during the latent period that often precedes the epileptic stage. Using the pilocarpine model of TLE in rats, we report that this ratio is decreased in hippocampal CA1 pyramidal cells during the early phase of the latent period (3–5 days postpilocarpine). It is, however, increased during the late phase of the latent period (7–10 days postpilocarpine), via cell domain-dependent alterations in synaptic current properties, concomitant with the occurrence of interictal-like activity in vivo. During the late latent period, the glutamatergic drive was increased in somata via an enhancement in EPSC decay time constant and in dendrites via an increase in EPSC frequency and amplitude. The GABAergic drive remained unchanged in the soma but was decreased in dendrites, since the drop off in IPSC frequency was more marked than the increase in IPSC kinetics. Theoretical considerations suggest that these modifications are sufficient to produce interictal-like activity. In epileptic animals, the ratio of the glutamatergic drive to the overall synaptic drive was not further modified, despite additional changes in synaptic current frequency and kinetics. These results show that the global changes to more glutamatergic and less GABAergic activities in the CA1 region precede the chronic stage of epilepsy, possibly facilitating the occurrence and/or the propagation of interictal activity.

COMMENTARY

The paper by El-Hassar and coworkers analyzed changes in excitatory (glutamatergic) and inhibitory (GABAergic) synaptic input that follows pilocarpine-induced status epilepticus; the study focused on reduced inhibitory postsynaptic currents (IPSCs) and increased excitatory postsynaptic currents (EPSCs) at the somata and apical dendrites during the early and late phases of the seizure-free latent period. The analysis of reduced inhibition is based on several lines of evidence from previous studies concerning the loss of specific types of GABAergic interneurons after kainate- or pilocarpine-induced status epilepticus (1,2). Similarly, the analysis of increased excitation relates to synaptic reorganization, likely arising from the onset of axonal sprouting and an increase in recurrent excitatory circuitry (3,4), which probably begins to occur within a few days after injury. The experiments used measurements of the amplitude, frequency, rise time, decay time, and charge transfer for both spontaneous IPSCs and EPSCs. By using whole-cell recording from visually identified pyramidal cells, the authors also were able to analyze the synaptic inputs to the dendrites versus the somata. Thus, these data describe the time-dependent changes in GABAergic and glutamatergic inputs to dendrites and somata during the early stages of epileptogenesis. The changes in the characteristics of the postsynaptic currents, as measured in vitro, reflect spontaneous release of transmitter from axons cut during brain slicing (i.e., presumed to have no action potentials); activity-dependent transmitter release from neurons present in the slice; and the effects of different synaptic inputs on those axon terminals and neurons in the recorded slice.

This paper aims to address the important conceptual issue of epileptogenesis, which is often defined as the changes in intrinsic and synaptic mechanisms that occur during the latent period between brain injury and the onset of spontaneous recurrent seizures. The authors have separated the latent period in the pilocarpine model into early and late epochs and have contrasted the data from these time epochs with the chronic phase of spontaneous recurrent seizures. As expected, the recordings reveal that changes are present within a few days after status epilepticus; further changes were detected within a few additional days, during the latent period before seizures generally begin. The data support the previous hypotheses that: (a) dendritic inhibition is reduced from the loss of dendritically projecting neurons, (b) somatic inhibition is increased from the loss of inhibitory input to somatically projecting interneurons, and (c) excitation is increased from formation of new recurrent excitatory circuits (5). As with many studies on synaptic reorganization, however, the data and the potential circuit mechanisms are more complicated than is immediately obvious.

The experiments by El-Hassar et al. focused on spontaneous IPSCs and EPSCs recorded in normal solutions, so that both inhibitory and excitatory mechanisms were pharmacologically intact. This protocol is in contrast to some other published reports in which GABAergic inhibition was studied by pharmacologically blocking glutamatergic excitation (6) or glutamatergic inputs were analyzed by blocking GABAergic transmission with $GABA_A$ and $GABA_B$ receptor antagonists (7). The former approach has the advantage that the tissue is more similar to the intact animal; however, the potential disadvantage is that the effects of epilepsy-associated alterations of one transmitter

Epilepsy Currents, Vol. 7, No. 3 (May/June) 2007 pp. 86–88
Blackwell Publishing, Inc.

system will impact the analysis of another system, and vice versa. For example, recordings of spontaneous EPSCs in CA1 pyramidal cells in normal solution reflect spontaneous transmitter release from all of the cut and uncut glutamatergic axons (i.e., miniature EPSCs) as well as from those glutamatergic neurons that are intact and spontaneously active. The activity of the latter neurons, in turn, will depend on their glutamatergic and GABAergic inputs, which may or may not be intact. This experimental system, therefore, is more complete but also more complicated than one in which the glutamatergic and GABAergic transmitter systems have been isolated. Thus, these experiments greatly reduce the complexity that is inherent in an intact animal preparation and also bypass the interpretational problems associated with experiments involving electrical stimulation, which are more complex than commonly appreciated.

A concept elaborated in this report is that GABA-mediated inhibition is decreased immediately after pilocarpine-induced status epilepticus, presumably as a result of the loss of specific interneurons (1–3) but also potentially because of other alterations arising from direct status-epilepticus–induced changes in $GABA_A$-receptor–mediated mechanisms. These changes, and potentially others, are hypothesized to lead to an increased propensity for generation of interictal spikes, which may be involved in the process of epileptogenesis (8). It is noteworthy that the isolated CA1 area does not typically generate spontaneous all-or-none epileptiform bursts reminiscent of interictal spikes in slices from normal animals treated with pharmacological agents that block $GABA_A$ receptors. The authors did not report bursts in any of the different types of slices (i.e., from sham controls; pilocarpine-treated during the latent period; or pilocarpine-induced chronically epileptic), although interictal spikes were recorded in freely behaving animals during the latent period before chronic spontaneous recurrent seizures (9). Although the authors included modeling studies that suggested that the detected alterations could account for the generation of interictal spikes, it also is possible that the generation of these events in the CA1 area could arise from abnormalities in other regions, such as the CA3 area, and be projected to the CA1 area. It is likely, therefore, that the changes described here—particularly the loss of GABAergic interneurons—occur in many areas, including the CA3 area, dentate gyrus, and entorhinal cortex and collectively could lead to the propensity to generate interical spikes, possibly over relatively large areas.

El-Hassar et al. found a particularly large increase in spontaneous glutamatergic input to the dendrites that was not evident in somatic recordings. While the origins of the differences between dendritic versus somatic recordings have been questioned, recent studies, using voltage-sensitive dye imaging technology, demonstrated a similar increase in glutamatergic input to the dendrites of CA1 pyramidal cells (10). These findings raise the possibility that key shifts in the balance between excitation and inhibition may take place in the dendrites. Dendritic alterations are particularly interesting because, as El-Hassar et al. speculate, the shift in balance of excitation and inhibition may well underlie the generation of interictal spikes and because the paroxysmal depolarizing shift in membrane potential (considered to underlie the interictal spike) has long been thought to depend on giant excitatory postsynaptic potentials and calcium spikes, both of which appear to originate in the dendrites.

The paper of El-Hassar and coworkers highlights several important points. First, analyses of synaptic inputs to hippocampal pyramidal cells (or any type of neuron) in tissue from an animal model of epilepsy can be quite complicated; although in vitro studies can simplify the analyses of synaptic mechanisms, the details of the experimental protocols may have important effects on the results. Second, a reduction of GABAergic input from a partial loss of specific interneurons is a common feature of the short-term effects of status epilepticus, and this effect has been detected in many studies as a reduction in the frequency of spontaneous IPSCs (and miniature IPSCs). Third, a reduction in $GABA_A$-mediated inhibition is a common method of inducing large and prolonged burst discharges (i.e., a few hundred milliseconds), which is a model for the interictal spike. Fourth, events that resemble prolonged synchronous bursts and interictal spikes often precede seizure-like events in vitro and frank seizures in vivo. The connections among these phenomena will require further investigations in order to link both interictal spikes and seizures associated with epilepsy to molecular, cellular, and network mechanisms.

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