Research on ionic homeostatic equilibrium may change our view about epilepsy

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Epilepsy is a diverse set of chronic neurological disorders characterized by seizures. Epilepsy affects individuals of all ages, races, social classes, geographic regions and nationalities (1-3). It is among the most common serious neurological conditions. In developed countries, epilepsy has a prevalence of approximately 1% (4,5). Each year, 24 per 100,000 people suffer from epilepsy in Europe and 53 per 100,000 in North America (5-7). In developing countries, the incidence is even higher, with a rate of up to 190 per 100,000 individuals (8,9). Furthermore, epilepsy can be considered a malignant condition because sudden death in individuals with epilepsy is estimated to be at least 20 times higher than in the general population (10,11).

The capacity to replicate human epilepsy in animal models is an important tool for experimental study. Animal studies have contributed significantly to the understanding of the biological basis of epileptogenesis and have provided evidence for the possible mechanisms of action of antiepileptic drugs. However, the relevance of animal models in human epilepsy research depends on how closely the model mimics the human condition (12). These models have provided important information on the brain and the behavioral mechanisms that could be involved in the etiology, pathophysiology and electrophysiological events and their correlations with synaptic interactions. However, the belief that the etiology of epilepsy can be traced to synaptic connections does not take into account the fact that the strength of synaptic interactions may change based on the intra- and extracellular ionic equilibrium.

The mechanisms involved in the intra- and extracellular regulation of ionic levels are usually ignored; however, it has been shown that neuronal and glial activities are intrinsically modulated by the ionic gradients through their cellular membranes. These gradients depend on the complex interaction of mechanisms related to ionic homeostatic regulation, such as the Na/K ATPase, cotransporters and exchanger enzymes. Furthermore, paroxysmal discharges are accompanied by significant changes in the intra- and extracellular ionic concentrations, which challenge the homeostatic equilibrium regulated by these mechanisms. Focally induced cortical seizures are preceded by small reductions in \([Ca^{2+}]_i\) that become intense during paroxysms (13,14). Posterior investigations (15,16) have clearly demonstrated that hippocampal slices exposed to low \([Ca^{2+}]_i\) are able to sustain non-synaptic epileptiform activity. Genetically epileptic baboons exhibited such significant drops in their \([Ca^{2+}]_i\) levels that all synaptic transmissions must have been blocked. However, the researchers did not observe any transmission disruptions in the course of the seizures (17). Overall, these data disprove the widely held belief that epileptic seizures are exclusively generated by the imbalance between excitation and inhibition.

Simultaneous findings showed that changes in the chloride transmembrane gradient might also occur and are able to modulate the activation of the gamma-aminobutyric acid A (GABA_A) receptors. These findings suggest that hyperpolarization or depolarization may occur in a manner dependent on intracellular chloride accumulation (18-21). The cation-chloride cotransporters and Cl\(^{-}/\)HCO\(_3\)\(^{-}\) exchanger were identified as the main regulators of the intracellular chloride concentration (22). In the mature brain, the low \([Cl^{-}]_i\) level is associated with a Cl^- Nernst potential that is more negative than the transmembrane potential; this results in Cl^- influx and a hyperpolarizing effect when GABA_A receptors are activated. In contrast, in the immature brain, the high intracellular Cl^- and positive Cl^- Nernst potential relative to the transmembrane potential cause a Cl^- efflux and a depolarizing inward current. Pathophysiological conditions, such as neuronal injuries and the inflammatory state, may also resemble the immature brain because of a decrease in the potassium chloride transporter KCC2 (23-26).

Based on this information, it is not difficult to surmise that changes in the extracellular concentration may also be accompanied by changes in the equilibrium of non-synaptic mechanisms. The extracellular K^- accumulation, which is always associated with intense neuronal firing, induces Cl^- intrusion through the cotransporters and, in turn, reinforces the increased excitation.

Because the synaptic circuit is part of a system in which non-synaptic mechanisms control ionic homeostasis, it is difficult to ignore the effect non-synaptic mechanisms have on seizure sustainment and progression. Therefore, our group has sought to investigate the effects that changes in non-synaptic mechanisms have on different experimental...
models of epilepsy. Due to the complexity, the first step of our investigation was to develop a computational model to understand the dynamics of the main mechanisms (27). The computational model has been extensively used in our group as an indispensable tool to guide our analysis of the electrophysiological data. Simulations representing the histological changes observed in the hippocampal slices are processed to understand how the changes in ionic concentrations support effective actions of the synaptic transmission and non-synaptic mechanisms to reduce seizures. Conversely, when the transmembrane ionic gradients are decreased (right), the synaptic transmissions are depressed and the interferences on the synaptic circuit are refractory. However, seizure reduction is expected when interfering with the non-synaptic mechanisms to restore ionic gradients.

**Figure 1** - Diagrammatic representation of conditions in which interference on synaptic transmission and/or non-synaptic mechanisms may affect seizures. Under normal ionic conditions, neuronal activity is not accompanied by important changes in the ionic concentrations (left). Therefore, the ionic gradients support effective actions of the synaptic transmission and non-synaptic mechanisms to reduce seizures. Conversely, when the transmembrane ionic gradients are decreased (right), the synaptic transmissions are depressed and the interferences on the synaptic circuit are refractory. However, seizure reduction is expected when interfering with the non-synaptic mechanisms to restore ionic gradients.
homeostasis may change the induced epileptiform activity (28). Our preliminary results show that despite the cellular death associated with the experimental models, the non-synaptic mechanisms are able to compensate for the loss and enhance the epileptiform activity sustained by the neuronal tissue. These promising first results open up new possibilities for understanding seizure disruption. It is also becoming clear that the mechanisms and conditions that disrupt and sustain seizures are highly complex. The evidence that non-synaptic mechanisms are able to modulate the function of the synaptic circuit indicates that the problem is even more complex than we suspected.

The simulations show that the typically intense ionic changes of the sites to which the paroxysmal neuronal population is recruited are able to reduce the corresponding transmembrane gradients of the ions to such a level that synaptic function is depressed. Because the main anti-epileptic drugs target synaptic functioning, no effect would be expected when the synapses are depressed. Therefore, these drugs would not act during the ictal period, nor would they act in epilepsies where the triggering condition is characterized by changes in ionic homeostasis, such as the intracellular Cl− accumulation typical of the immature brain, brain injury and brain inflammation (Figure 1).

Finally, we believe that this is the first step in a long scientific journey that will trigger new research and debates. Thus, it is crucial to promote scientific collaboration to investigate non-synaptic mechanisms of epilepsies and to discover promising drugs that act non-synthetically. This new and exciting possibility for epilepsy research makes us reflect on this quote by Galileo Galilei:

“Every new and exciting possibility for epilepsy research makes us discover promising drugs that act non-synthetically. This allows us to promote scientific collaboration to investigate non-synaptic mechanisms of epilepsies and to discover promising drugs that act non-synthetically. This new and exciting possibility for epilepsy research makes us reflect on this quote by Galileo Galilei: “The Bible shows the way to go to heaven, not the way the heavens go.”

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